```
FILE 'HOME' ENTERED AT 18:04:49 ON 12 MAY 2003)

     FILE 'STNGUIDE' ENTERED AT 18:05:11 ON 12 MAY 2003

     FILE 'HOME' ENTERED AT 18:05:20 ON 12 MAY 2003

     FILE 'MEDLINE' ENTERED AT 18:05:31 ON 12 MAY 2003
                E CHEN J M/AU 25
L1            209 S (E3) AND 1990<=PY<=2003
L2             31 S (E3) AND 2001<=PY<=2003
                E BARRET A J/AU 25
L3              0 S (E3) AND 2001<=PY<=2003
                E RAWLINGS N D/AU 25
L4              2 S (E3) AND 2001<=PY<=2003

     FILE 'MEDLINE' ENTERED AT 18:23:29 ON 12 MAY 2003
L5              0 S NEUROLYSIN (A) HUMAN
L6             48 S NEUROLYSIN
L7              0 S L6 (A) GENE
L8              0 S L6 (A)CLONING
L9              0 S L6 (A) CLONING
L10             0 S NEUROLYSISN GENE
L11             0 S NEUROLYSINE (A)SEQUENCE
L12             0 S NEUROLYSIN (A) GENE
L13             0 S NEUROLYSIN (A) DNA
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FILE 'HOME' ENTERED AT 18:04:49 ON 12 MAY 2003)

     FILE 'STNGUIDE' ENTERED AT 18:05:11 ON 12 MAY 2003

     FILE 'HOME' ENTERED AT 18:05:20 ON 12 MAY 2003

     FILE 'MEDLINE' ENTERED AT 18:05:31 ON 12 MAY 2003
                E CHEN J M/AU 25
L1            209 S (E3) AND 1990<=PY<=2003
L2             31 S (E3) AND 2001<=PY<=2003
                E BARRET A J/AU 25
L3              0 S (E3) AND 2001<=PY<=2003
                E RAWLINGS N D/AU 25
L4              2 S (E3) AND 2001<=PY<=2003

     FILE 'MEDLINE' ENTERED AT 18:23:29 ON 12 MAY 2003
L5              0 S NEUROLYSIN (A) HUMAN
L6             48 S NEUROLYSIN
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L6 ANSWER 1 OF 48 MEDLINE
AN 2003200463 IN-PROCESS
DN 22592979 PubMed ID: 12706825
TI A structure-based site-directed mutagenesis study on the **neurolysin** (EC 3.4.24.16) and thimet oligopeptidase (EC 3.4.24.15) catalysis.
AU Oliveira Vitor; Araujo Mauricio C; Rioli Vanessa; de Camargo Antonio C M; Tersariol Ivarne L S; Juliano Maria A; Juliano Luiz; Ferro Emer S
CS Departamento de Biofisica, Universidade Federal de Sao Paulo, Brazil.. vitor.biof@infar.epm.br
SO FEBS LETTERS, (2003 Apr 24) 541 (1-3) 89-92.
Journal code: 0155157. ISSN: 0014-5793.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS IN-PROCESS; NONINDEXED; Priority Journals
ED Entered STN: 20030501
Last Updated on STN: 20030501
AB **Neurolysin** (EP24.16) and thimet oligopeptidase (EP24.15) are closely related metalloendopeptidases. Site-directed mutagenesis of Tyr(613) (EP24.16) or Tyr(612) (EP24.15) to either Phe or Ala promoted a strong reduction of k(cat)/K(M) for both enzymes. These data suggest the importance of both hydroxyl group and aromatic ring at this specific position during substrate hydrolysis by these peptidases. Furthermore, the EP24.15 A607G mutant showed a k(cat)/K(M) of 2x10(5) M(-1) s(-1) for the Abz-GFSIFRQ-EDDnp substrate, similar to that of EP24.16 (k(cat)/K(M)=3x10(5) M(-1) s(-1)) which contains Gly at the corresponding position; the wild type EP24.15 has a k(cat)/K(M) of 2.5x10(4) M(-1) s(-1)
for this substrate.

L6 ANSWER 2 OF 48 MEDLINE
AN 2003114320 MEDLINE
DN 22499638 PubMed ID: 12500972
TI Novel natural peptide substrates for endopeptidase 24.15, **neurolysin**, and angiotensin-converting enzyme.
AU Rioli Vanessa; Gozzo Fabio C; Heimann Andrea S; Linardi Alessandra; Krieger Jose E; Shida Claudio S; Almeida Paulo C; Hyslop Stephen; Eberlin Marcos N; Ferro Emer S
CS Department of Histology and Embryology, Cell Biology Program, Institute of
Biomedical Sciences, University of Sao Paulo, Brazil.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2003 Mar 7) 278 (10) 8547-55.
Journal code: 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200304
ED Entered STN: 20030312
Last Updated on STN: 20030424
Entered Medline: 20030423
AB Endopeptidase 24.15 (EC; ep24.15), **neurolysin** (EC; ep24.16), and angiotensin-converting enzyme (EC; ACE) are metallopeptidases involved in neuropeptide metabolism in vertebrates. Using catalytically inactive forms of ep24.15 and ep24.16, we have identified new peptide substrates for these enzymes. The enzymatic activity of ep24.15 and ep24.16 was inactivated by site-directed mutagenesis of amino acid residues within their conserved HEXXH motifs, without disturbing their secondary structure

or peptide binding ability, as shown by circular dichroism and binding assays. Fifteen of the peptides isolated were sequenced by electrospray ionization tandem mass spectrometry and shared homology with fragments of intracellular proteins such as hemoglobin. Three of these peptides (PVNFKFLSH, VVYPWTQRY, and LVVYPWTQRY) were synthesized and shown to interact with ep24.15, ep24.16, and ACE, with K(i) values ranging from 1.86 to 27.76 microm. The hemoglobin alpha-chain fragment PVNFKFLSH, which we have named hemopressin, produced dose-dependent hypotension in anesthetized rats, starting at 0.001 microg/kg. The hypotensive effect of the peptide was potentiated by enalapril only at the lowest peptide dose. These results suggest a role for hemopressin as a vasoactive substance in vivo. The identification of these putative intracellular substrates for ep24.15 and ep24.16 is an important step toward the elucidation of the role of these enzymes within cells.

CT Check Tags: Animal; Male; Support, Non-U.S. Gov't
Amino Acid Sequence
Chromatography, High Pressure Liquid
Circular Dichroism
Electrophoresis, Polyacrylamide Gel
Hemoglobins: CH, chemistry
*Hemoglobins: ME, metabolism
Hemoglobins: PH, physiology
Metalloendopeptidases: GE, genetics
*Metalloendopeptidases: ME, metabolism
Molecular Sequence Data
Mutagenesis, Site-Directed
Peptide Fragments: CH, chemistry
*Peptide Fragments: ME, metabolism
Peptide Fragments: PH, physiology
*Peptidyl-Dipeptidase A: ME, metabolism
Rats
Rats, Wistar
Spectrometry, Mass, Electrospray Ionization
Substrate Specificity
Swine

CN 0 (Hemoglobins); 0 (Peptide Fragments); 0 (hemopressin); EC 3.4.15.1 (Peptidyl-Dipeptidase A); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.15 (thimet oligopeptidase); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 3 OF 48 MEDLINE
AN 2003047536 MEDLINE
DN 22444752 PubMed ID: 12540854
TI Crystal structure of the human angiotensin-converting enzyme-lisinopril complex.
AU Natesh Ramanathan; Schwager Sylva L U; Sturrock Edward D; Acharya K Ravi
CS Department of Biology and Biochemistry, University of Bath, Claverton Down, Bath BA2 7AY, UK.
SO NATURE, (2003 Jan 30) 421 (6922) 551-4.
Journal code: 0410462. ISSN: 0028-0836.
CY England: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS PDB-1O86; PDB-1O8A
EM 200303
ED Entered STN: 20030131
Last Updated on STN: 20030305
Entered Medline: 20030304
AB Angiotensin-converting enzyme (ACE) has a critical role in cardiovascular

function by cleaving the carboxy terminal His-Leu dipeptide from angiotensin I to produce a potent vasopressor octapeptide, angiotensin II.

Inhibitors of ACE are a first line of therapy for hypertension, heart failure, myocardial infarction and diabetic nephropathy. Notably, these inhibitors were developed without knowledge of the structure of human ACE,

but were instead designed on the basis of an assumed mechanistic homology with carboxypeptidase A. Here we present the X-ray structure of human testicular ACE and its complex with one of the most widely used inhibitors, lisinopril (N2-[(S)-1-carboxy-3-phenylpropyl]-L-lysyl-L-proline; also known as Prinivil or Zestril), at 2.0 A resolution. Analysis of the three-dimensional structure of ACE shows that it bears little similarity to that of carboxypeptidase A, but instead resembles **neurolysin** and Pyrococcus furiosus carboxypeptidase--zinc metallopeptidases with no detectable sequence similarity to ACE. The structure provides an opportunity to design domain-selective ACE inhibitors that may exhibit new pharmacological profiles.

CT Check Tags: Human; Male; Support, Non-U.S. Gov't
Amino Acid Sequence
*Angiotensin-Converting Enzyme Inhibitors: CH, chemistry
*Angiotensin-Converting Enzyme Inhibitors: ME, metabolism
Binding Sites
Carboxypeptidases: CH, chemistry
Carboxypeptidases: ME, metabolism
Crystallography, X-Ray
Drug Design
*Lisinopril: CH, chemistry
*Lisinopril: ME, metabolism
Metalloendopeptidases: CH, chemistry
Metalloendopeptidases: ME, metabolism
Models, Molecular
Molecular Sequence Data
*Peptidyl-Dipeptidase A: CH, chemistry
*Peptidyl-Dipeptidase A: ME, metabolism
Protein Conformation
Pyrococcus furiosus: EN, enzymology
Substrate Specificity
RN 83915-83-7 (Lisinopril)
CN 0 (Angiotensin-Converting Enzyme Inhibitors); EC 3.4.- (Carboxypeptidases); EC 3.4.15.1 (Peptidyl-Dipeptidase A); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 4 OF 48 MEDLINE
AN 2002615573 MEDLINE
DN 22259839 PubMed ID: 12372844
TI Soluble metalloendopeptidases and neuroendocrine signaling.
AU Shrimpton Corie N; Smith A Ian; Lew Rebecca A
CS Baker Medical Research Institute, Melbourne, Australia 8008.
SO ENDOCRINE REVIEWS, (2002 Oct) 23 (5) 647-64. Ref: 162
Journal code: 8006258. ISSN: 0163-769X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Priority Journals
EM 200303
ED Entered STN: 20021010
Last Updated on STN: 20030305

Entered Medline: 20030304
AB Peptidases play a vital and often highly specific role in the physiological and pathological generation and termination of peptide hormone signals. The thermolysin-like family of metalloendopeptidases involved in the extracellular processing of neuroendocrine and cardiovascular peptides are of particular significance, reflecting both their specificity for particular peptide substrates and their utility as therapeutic targets. Although the functions of the membrane-bound members of this family, such as angiotensin-converting enzyme and neutral endopeptidase, are well established, a role for the predominantly soluble family members in peptide metabolism is only just emerging. This review will focus on the biochemistry, cell biology, and physiology of the soluble metalloendopeptidases EC 3.4.24.15 (thimet oligopeptidase) and EC 3.4.24.16 (**neurolysin**), as well as presenting evidence that both peptidases play an important role in such diverse functions as reproduction, nociception, and cardiovascular homeostasis.
CT Check Tags: Animal
Amino Acid Sequence
Binding Sites
Cardiovascular System: EN, enzymology
Immunohistochemistry
Metalloendopeptidases: AN, analysis
Metalloendopeptidases: CH, chemistry
*Metalloendopeptidases: PH, physiology
Models, Molecular
Molecular Sequence Data
*Neurosecretory Systems
Neurosecretory Systems: EN, enzymology
Sequence Alignment
*Signal Transduction
Solubility
Substrate Specificity
Tissue Distribution
CN EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.15 (thimet oligopeptidase); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 5 OF 48 MEDLINE
AN 2002491841 MEDLINE
DN 22188683 PubMed ID: 12199711
TI Temperature and salts effects on the peptidase activities of the recombinant metallooligopeptidases **neurolysin** and thimet oligopeptidase.
AU Oliveira Vitor; Gatti Reynaldo; Rioli Vanessa; Ferro Emer S; Spisni Alberto; Camargo Antonio C M; Juliano Maria A; Juliano Luiz
CS Department of Biophysics, Escola Paulista de Medicina, Sao Paulo, Brazil.
SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (2002 Sep) 269 (17) 4326-34. Journal code: 0107600. ISSN: 0014-2956.
CY Germany: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200211
ED Entered STN: 20021001
Last Updated on STN: 20021213
Entered Medline: 20021105
AB We report the recombinant **neurolysin** and thimet oligopeptidase (TOP) hydrolytic activities towards internally quenched fluorescent peptides derived from the peptide Abz-GGFLRRXQ-EDDnp (Abz,

ortho-aminobenzoicacid; EDDnp, N-(2,4-dinitrophenyl) ethylenediamine), in which X was substituted by 11 different natural amino acids. **Neurolysin** hydrolyzed these peptides at R-R or at R-X bonds, and TOP hydrolyzed at R-R or L-R bonds, showing a preference to cleave at three or four amino acids from the C-terminal end. The kinetic parameters of hydrolysis and the variations of the cleavage sites were evaluated under different conditions of temperature and salt concentration. The relative amount of cleavage varied with the nature of the substitution at the X position as well as with temperature and NaCl concentration. TOP was activated by all assayed salts in the range 0.05-0.2 m for NaCl, KCl, NH4Cl and NaI, and 0.025-0.1 m for Na2SO4. Concentration higher than 0.2 N NH4Cl and NaI reduced TOP activity, while 0.5 N or higher concentration of NaCl, KCl and Na2SO4 increased TOP activity. **Neurolysin** was strongly activated by NaCl, KCl and Na2SO4, while NH4Cl and NaI have very modest effect. High positive values of enthalpy (DeltaH*) and entropy (DeltaS*) of activation were found together with an unusual temperature dependence upon the hydrolysis of the substrates. The effects of low temperature and high NaCl concentration on the hydrolytic activities of **neurolysin** and TOP do not seem to be a consequence of large secondary structure variation of the proteins, as indicated by the far-UV CD spectra. However, the modulation of the activities of the two oligopeptidases could be related to variations of conformation, in limited regions of the peptidases, enough to modify their activities.

CT Check Tags: Animal; Comparative Study; Male; Support, Non-U.S. Gov't
Amino Acids: AN, analysis
Circular Dichroism
Enzyme Stability
Heat
Hydrolysis
Kinetics
Liver: EN, enzymology
Metalloendopeptidases: CH, chemistry
*Metalloendopeptidases: ME, metabolism
Metals: CH, chemistry
Metals: ME, metabolism
Oligopeptides: CS, chemical synthesis
*Oligopeptides: ME, metabolism
Protease Inhibitors: PD, pharmacology
Rats
Recombinant Proteins: ME, metabolism
Sodium Chloride: PD, pharmacology
Substrate Specificity
Swine
Testis: EN, enzymology

RN 7647-14-5 (Sodium Chloride)

CN 0 (Amino Acids); 0 (Metals); 0 (Oligopeptides); 0 (Protease Inhibitors); 0 (Recombinant Proteins); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.15 (thimet oligopeptidase); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 6 OF 48 MEDLINE

AN 2002451113 MEDLINE

DN 22183961 PubMed ID: 12196021

TI Inhibitors of metalloendopeptidase EC 3.4.24.15 and EC 3.4.24.16 stabilized against proteolysis by the incorporation of beta-amino acids.

AU Steer David; Lew Rebecca; Perlmutter Patrick; Smith A Ian; Aguilar Marie-Isabel

CS Department of Biochemistry and Molecular Biology, P.O. Box 13D, Monash

University, Clayton, Vic 3800, Australia.
SO BIOCHEMISTRY, (2002 Sep 3) 41 (35) 10819-26.
Journal code: 0370623. ISSN: 0006-2960.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200210
ED Entered STN: 20020906
Last Updated on STN: 20021002
Entered Medline: 20021001
AB The enzyme EC 3.4.24.15 (EP 24.15) is a zinc metalloendopeptidase whose precise function in vivo remains unknown but is thought to participate in the regulated metabolism of a number of specific neuropeptides. The lack of stable and selective inhibitors has hindered the determination of the exact function of EP 24.15. Of the limited number of EP 24.15 inhibitors that have been developed, N-[1-(R,S)-carboxy-3-phenylpropyl]-Ala-Ala-Tyr-p-aminobenzoate (CFP) is the most widely studied. CFP is a potent and specific inhibitor, but it is unstable in vivo due to cleavage between the alanine and tyrosine residues by the enzyme neprilysin (EP 24.11). This cleavage by EP 24.11 generates a potent inhibitor of angiotensin converting enzyme, thereby limiting the use of CFP for in vivo studies. To develop specific inhibitors of EP 24.15 that are resistant to in vitro and potentially in vivo proteolysis by EP 24.11, this study incorporated beta-amino acids replacing the Ala-Tyr scissile alpha-amino acids of CFP. Both C2 and C3 substituted beta-amino acids were synthesized and substituted at the EP 24.11 scissile Ala-Tyr bond. Significant EP 24.15 inhibitory activity was observed with some of the beta-amino acid containing analogues. Moreover, binding to EP 24.11 was eliminated, thus rendering all analogues containing beta-amino acids resistant to degradation by EP 24.11. Selective inhibition of either EP 24.15 or EP 24.16 was also observed with some analogues. The results demonstrated the use of beta-amino acids in the design of inhibitors of EP 24.15 and EP 24.16 with K(i)'s in the low micromolar range. At the same time, these analogues were resistant to cleavage by the related metalloendopeptidase EP 24.11, in contrast to the alpha-amino acid based parent peptide. This study has therefore clearly shown the potential of beta-amino acids in the design of stable enzyme inhibitors and their use in generating molecules with selectivity between closely related enzymes.
CT Check Tags: Animal
Amino Acid Substitution
*Amino Acids: ME, metabolism
Drug Stability
Glycine: ME, metabolism
Hydrolysis
*Metalloendopeptidases: AI, antagonists & inhibitors
*Metalloendopeptidases: ME, metabolism
Molecular Mimicry
Neprilysin: CH, chemistry
Neprilysin: ME, metabolism
Oligopeptides: CS, chemical synthesis
Oligopeptides: CH, chemistry
Oligopeptides: ME, metabolism
Peptide Synthesis
*Proline: AA, analogs & derivatives
Proline: ME, metabolism

Protease Inhibitors: CS, chemical synthesis
*Protease Inhibitors: CH, chemistry
Protease Inhibitors: ME, metabolism
Rats
Structure-Activity Relationship
Tyrosine: ME, metabolism
beta-Alanine: ME, metabolism
RN 107-95-9 (beta-Alanine); 147-85-3 (Proline); 55520-40-6 (Tyrosine); 56-40-6 (Glycine); 59378-87-9 (beta-proline)
CN 0 (Amino Acids); 0 (JA 2 compound); 0 (Oligopeptides); 0 (Protease Inhibitors); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.11 (Neprilysin); EC 3.4.24.15 (thimet oligopeptidase); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 7 OF 48 MEDLINE
AN 2002435011 MEDLINE
DN 22180142 PubMed ID: 12192079
TI Mapping sequence differences between thimet oligopeptidase and **neurolysin** implicates key residues in substrate recognition.
AU Ray Kallol; Hines Christina S; Rodgers David W
CS Department of Molecular and Cellular Biochemistry and Center for Structural Biology, University of Kentucky, Lexington, Kentucky 40536, USA.
NC CA14596 (NCI)
NS38041 (NINDS)
SO PROTEIN SCIENCE, (2002 Sep) 11 (9) 2237-46.
Journal code: 9211750. ISSN: 0961-8368.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200303
ED Entered STN: 20020823
Last Updated on STN: 20030305
Entered Medline: 20030304
AB The highly homologous endopeptidases thimet oligopeptidase and **neurolysin** are both restricted to short peptide substrates and share many of the same cleavage sites on bioactive and synthetic peptides.
They sometimes target different sites on the same peptide, however, and defining the determinants of differential recognition will help us to understand how both enzymes specifically target a wide variety of cleavage
site sequences. We have mapped the positions of the 224 surface residues that differ in sequence between the two enzymes onto the surface of the **neurolysin** crystal structure. Although the deep active site channel accounts for about one quarter of the total surface area, only 11%
of the residue differences map to this region. Four isolated sequence changes (R470/E469, R491/M490, N496/H495, and T499/R498; **neurolysin** residues given first) are well positioned to affect recognition of substrate peptides, and differences in cleavage site specificity can be largely rationalized on the basis of these changes. We
also mapped the positions of three cysteine residues believed to be responsible for multimerization of thimet oligopeptidase, a process that inactivates the enzyme. These residues are clustered on the outside of one channel wall, where multimerization via disulfide formation is unlikely to block the substrate-binding site. Finally, we mapped the regulatory phosphorylation site in thimet oligopeptidase to a location on

the outside of the molecule well away from the active site, which indicates this modification has an indirect effect on activity.

CT Check Tags: Animal; Support, U.S. Gov't, Non-P.H.S.; Support, U.S. Gov't, P.H.S.
Amino Acid Sequence
Binding Sites
*Metalloendopeptidases: CH, chemistry
Metalloendopeptidases: ME, metabolism
Models, Molecular
Molecular Sequence Data
Peptide Mapping
Protein Conformation
*Protein Structure, Tertiary
Rats
Sequence Alignment
*Sequence Analysis, Protein
Substrate Specificity

CN EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.15 (thimet oligopeptidase); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 8 OF 48 MEDLINE

AN 2002217112 MEDLINE

DN 21950684 PubMed ID: 11809755

TI Insulin-degrading enzyme rapidly removes the beta-amyloid precursor protein intracellular domain (AICD).

AU Edbauer Dieter; Willem Michael; Lammich Sven; Steiner Harald; Haass Christian

CS Department of Biochemistry, Laboratory for Alzheimer's and Parkinson's Disease Research, Adolf-Butenandt-Institute, Ludwig-Maximilians-University, Schillerstrasse 44, 80336 Munich, Germany.

SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2002 Apr 19) 277 (16) 13389-93. Journal code: 2985121R. ISSN: 0021-9258.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 200206

ED Entered STN: 20020416
Last Updated on STN: 20030105
Entered Medline: 20020607

AB The intramembranous gamma-secretase cleavage of the beta-amyloid precursor protein (APP) is dependent on biologically active presenilins (PS). Notch also undergoes a similar PS-dependent gamma-secretase-like cleavage, resulting in the liberation of the Notch intracellular domain (NICD), which is critically required for developmental signal transduction. gamma-Secretase processing of APP results in the production of a similar fragment called AICD (APP intracellular domain), which may function in nuclear signaling as well. AICD, like NICD, is rapidly removed. By using a battery of protease inhibitors we demonstrate that AICD, in contrast to NICD, is degraded by a cytoplasmic metalloprotease. In vitro degradation of AICD can be reconstituted with cytoplasmic fractions obtained from neuronal and non-neuronal cells. Taking into account the inhibition profile and the cytoplasmic localization, we identified three candidate enzymes (**neurolysin**, thimet oligopeptidase, and insulin-degrading enzyme (IDE), also known as insulysin), which all are involved in the degradation of bioactive peptides in the brain. When insulin, a well characterized substrate of IDE, was added to the in vitro

degradation assay, removal of AICD was efficiently blocked. Moreover, overexpression of IDE resulted in enhanced degradation of AICD, whereas overexpression of the inactive IDE E111Q mutant did not affect AICD degradation. Finally, immunodepletion of IDE significantly reduced the AICD degrading activity. Therefore our data demonstrate that IDE, which is one of the proteases implicated in the removal of extracellular Abeta, also removes the cytoplasmic product of gamma-secretase cleaved APP.

CT Check Tags: Animal; Human; Support, Non-U.S. Gov't
*Amyloid beta-Protein Precursor: CH, chemistry
Cell Line
Cell Nucleus: ME, metabolism
Cytoplasm: CH, chemistry
Cytosol: EN, enzymology
Cytosol: ME, metabolism
DNA, Complementary: ME, metabolism
*Insulysin: CH, chemistry
Mice
Models, Biological
Protein Structure, Tertiary
Rats
Signal Transduction
Transfection
Tumor Cells, Cultured

CN 0 (Amyloid beta-Protein Precursor); 0 (DNA, Complementary); EC 3.4.24.56 (Insulysin)

L6 ANSWER 9 OF 48 MEDLINE
AN 2002140798 MEDLINE
DN 21829592 PubMed ID: 11839307
TI Crystal structure of a novel carboxypeptidase from the hyperthermophilic archaeon Pyrococcus furiosus.
AU Arndt Joseph W; Hao Bing; Ramakrishnan Vijay; Cheng Timothy; Chan Sunney I; Chan Michael K
CS Department of Chemistry, The Ohio State University, 484 West 12th Avenue, Columbus, OH 43210, USA.
NC GM 22432 (NIGMS)
GM 61796 (NIGMS)
RR07707 (NCRR)
T32 GM 08512 (NIGMS)
SO Structure (Camb), (2002 Feb) 10 (2) 215-24.
Journal code: 101087697. ISSN: 0969-2126.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS PDB-1K9X; PDB-1KA2; PDB-1KA4
EM 200205
ED Entered STN: 20020307
Last Updated on STN: 20020508
Entered Medline: 20020507
AB The structure of Pyrococcus furiosus carboxypeptidase (PfuCP) has been determined to 2.2 A resolution using multiwavelength anomalous diffraction (MAD) methods. PfuCP represents the first structure of the new M32 family of carboxypeptidases. The overall structure is comprised of a homodimer. Each subunit is mostly helical with its most pronounced feature being a deep substrate binding groove. The active site lies at the bottom of this groove and contains an HEXXH motif that coordinates the metal ion required

for catalysis. Surprisingly, the structure is similar to the recently reported rat **neurolysin**. Comparison of these structures as well as sequence analyses with other homologous proteins reveal several conserved residues. The roles for these conserved residues in the catalytic mechanism are inferred based on modeling and their location.

CT Check Tags: Animal; Support, Non-U.S. Gov't; Support, U.S. Gov't, Non-P.H.S.; Support, U.S. Gov't, P.H.S.
Amino Acid Sequence
Binding Sites
*Carboxypeptidases: CH, chemistry
Carboxypeptidases: ME, metabolism
Catalysis
Crystallography, X-Ray
Electrostatics
Isoenzymes: CH, chemistry
Isoenzymes: ME, metabolism
Metalloendopeptidases: CH, chemistry
Models, Molecular
Molecular Sequence Data
Protein Binding
Protein Conformation
*Pyrococcus furiosus: EN, enzymology
Sequence Homology, Amino Acid
Stereoisomerism
Structure-Activity Relationship

CN 0 (Isoenzymes); EC 3.4.- (Carboxypeptidases); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 10 OF 48 MEDLINE
AN 2002007179 MEDLINE
DN 21145809 PubMed ID: 11248043
TI Structure of **neurolysin** reveals a deep channel that limits substrate access.
AU Brown C K; Madauss K; Lian W; Beck M R; Tolbert W D; Rodgers D W
CS Department of Molecular and Cellular Biochemistry and Center for Structural Biology, University of Kentucky, Lexington, KY 40536, USA.
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (2001 Mar 13) 98 (6) 3127-32.
Journal code: 7505876. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS PDB-1I1I
EM 200112
ED Entered STN: 20020121
Last Updated on STN: 20030105
Entered Medline: 20011204

AB The zinc metallopeptidase **neurolysin** is shown by x-ray crystallography to have large structural elements erected over the active site region that allow substrate access only through a deep narrow channel. This architecture accounts for specialization of this neuropeptidase to small bioactive peptide substrates without bulky secondary and tertiary structures. In addition, modeling studies indicate that the length of a substrate N-terminal to the site of hydrolysis is restricted to approximately 10 residues by the limited size of the active site cavity. Some structural elements of **neurolysin**, including a five-stranded beta-sheet and the two active site helices, are conserved with other metallopeptidases. The connecting loop regions of these

elements, however, are much extended in **neurolysin**, and they, together with other open coil elements, line the active site cavity. These potentially flexible elements may account for the ability of the enzyme to cleave a variety of sequences.

CT Check Tags: Support, U.S. Gov't, Non-P.H.S.
Binding Sites
Crystallography, X-Ray
*Metalloendopeptidases: CH, chemistry
Models, Molecular
Protein Structure, Tertiary
Substrate Specificity

CN EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 11 OF 48 MEDLINE
AN 2001511578 MEDLINE
DN 21443836 PubMed ID: 11559896
TI Comparative fine structural distribution of endopeptidase 24.15 (EC3.4.24.15) and 24.16 (EC3.4.24.16) in rat brain.
AU Fontenele-Neto J D; Massarelli E E; Gurgel Garrido P A; Beaudet A; Ferro E
S
CS Department of Histology and Embryology, Cell Biology Program, Biomedical Sciences Institute, USP, Sao Paulo 05508-900, SP, Brazil.
SO JOURNAL OF COMPARATIVE NEUROLOGY, (2001 Oct 1) 438 (4) 399-410.
Journal code: 0406041. ISSN: 0021-9967.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200110
ED Entered STN: 20010918
Last Updated on STN: 20011029
Entered Medline: 20011025

AB Endopeptidase 24.15 (EP24.15) and 24.16 (EP24.16) are closely related metalloendopeptidases implicated in the metabolism of several neuropeptides and widely expressed in mammalian brain. To gain insight into the functional role of these two enzymes in the central nervous system, we examined their cellular and subcellular distribution in rat brain by using electron microscopic immunogold labeling. In all areas examined, EP24.15 and EP24.16 immunoreactivity were observed in selective subpopulations of neuronal and glial cells. Subcellular localization of EP24.15 in neurons revealed that this enzyme was predominantly concentrated in the nucleus, whereas EP24.16 was almost exclusively cytoplasmic. The amount of EP24.15 found in the nucleus was inversely correlated with that found in the cytoplasm, suggesting that the enzyme could be mobilized from one compartment to the other. Within the cytoplasm, EP24.15 and EP24.16 immunoreactivity showed comparable distributional patterns. Both enzymes were detected throughout perikarya and dendrites, as well as within axons and axon terminals. In all neuronal compartments, EP24.15 and EP24.16 showed a major association with
membranes of neurosecretory elements, including Golgi cisternae, tubulovesicular organelles, synaptic vesicles, and endosomes. However, whereas EP24.15 always faced the cytoplasmic face of the membranes, EP24.16 was observed on both cytoplasmic and luminal sides, suggesting that the latter was more likely to contribute to the processing of peptides or to the degradation of internalized ligands. Taken together, the present results suggest that EP24.15 could play a major role in the hydrolysis of intranuclear substrates, whereas EP24.16 would be predominantly involved in the processing and inactivation of signaling

peptides.
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CT Check Tags: Animal; Comparative Study; Male; Support, Non-U.S. Gov't
*Brain: EN, enzymology
Brain: UL, ultrastructure
Cell Compartmentation: PH, physiology
Cell Nucleus Structures: EN, enzymology
Cell Nucleus Structures: UL, ultrastructure
Cerebellar Cortex: EN, enzymology
Cerebellar Cortex: UL, ultrastructure
Cerebral Cortex: EN, enzymology
Cerebral Cortex: UL, ultrastructure
Cytoskeleton: EN, enzymology
Cytoskeleton: UL, ultrastructure
Dendrites: EN, enzymology
Dendrites: UL, ultrastructure
Immunohistochemistry
Intracellular Membranes: EN, enzymology
Intracellular Membranes: UL, ultrastructure
*Metalloendopeptidases: ME, metabolism
Microscopy, Electron
*Neuroglia: EN, enzymology
Neuroglia: UL, ultrastructure
*Neurons: EN, enzymology
Neurons: UL, ultrastructure
*Neuropeptides: ME, metabolism
Organelles: EN, enzymology
Organelles: UL, ultrastructure
Presynaptic Terminals: EN, enzymology
Presynaptic Terminals: UL, ultrastructure
Rats
Rats, Wistar
Solitary Nucleus: EN, enzymology
Solitary Nucleus: UL, ultrastructure
CN 0 (Neuropeptides); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.15 (thimet
oligopeptidase); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 12 OF 48 MEDLINE
AN 2001264048 MEDLINE
DN 21255186 PubMed ID: 11355859
TI Selective neurotensin-derived internally quenched fluorogenic substrates for **neurolysin** (EC 3.4.24.16): comparison with thimet oligopeptidase (EC 3.4.24.15) and neprilysin (EC 3.4.24.11).
AU Oliveira V; Campos M; Hemerly J P; Ferro E S; Camargo A C; Juliano M A; Juliano L
CS Department of Biophysics, Escola Paulista de Medicina, Universidade Federal de Sao Paulo, Rua Tres de Maio, 100, Sao Paulo, SP, 04044-020, Brazil.
SO ANALYTICAL BIOCHEMISTRY, (2001 May 15) 292 (2) 257-65.
Journal code: 0370535. ISSN: 0003-2697.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200107
ED Entered STN: 20010723
Last Updated on STN: 20010723
Entered Medline: 20010719
AB Internally quenched fluorescent peptides derived from neurotensin

(pELYENKPRRPYIL) sequence were synthesized and assayed as substrates for **neurolysin** (EC 3.4.24.16), thimet oligopeptidase (EC 3.4.24.15 or TOP), and neprilysin (EC 3.4.24.11 or NEP). Abz-LYENKPRRPYILQ-EDDnp (where EDDnp is N-(2,4-dinitrophenyl)ethylenediamine and Abz is ortho-aminobenzoic acid) was derived from neurotensin by the introduction of Q-EDDnp at the C-terminal end of peptide and by the substitution of the pyroglutamic (pE) residue at N-terminus for Abz and a series of shorter peptides was obtained by deletion of amino acids residues from C-terminal, N-terminal, or both sides. **Neurolysin** and TOP hydrolyzed the substrates at P--Y or Y--I or R--R bonds depending on the sequence and size of the peptides, while NEP cleaved P-Y or Y-I bonds according to its S'(1) specificity. One of these substrates, Abz-NKPRRPQ-EDDnp was a specific and sensitive substrate for **neurolysin** (k(cat) = 7.0 s(-1), K(m) = 1.19 microM and k(cat)/K(m) = 5882 mM(-1). s(-1)), while it was completely resistant to NEP and poorly hydrolyzed by TOP and also by prolyl oligopeptidase (EC 3.4.21.26). **Neurolysin** concentrations as low as 1 pM were detected using this substrate under our conditions and its analogue Abz-NKPRAPQ-EDDnp was hydrolyzed by **neurolysin** with k(cat) = 14.03 s(-1), K(m) = 0.82 microM, and k(cat)/K(m) = 17,110 mM(-1). s(-1), being the best substrate so far described for this peptidase.

CT Check Tags: Comparative Study; Human; Support, Non-U.S. Gov't
Alanine: GE, genetics
Alanine: ME, metabolism
Amino Acid Sequence
Fluorescent Dyes: CH, chemistry
*Fluorescent Dyes: ME, metabolism
Hydrolysis
Kinetics
*Metalloendopeptidases: ME, metabolism
Mutation: GE, genetics
*Neprilysin: ME, metabolism
*Neurotensin: AA, analogs & derivatives
Neurotensin: CH, chemistry
*Neurotensin: ME, metabolism
Sensitivity and Specificity
Serine Endopeptidases: ME, metabolism
Substrate Specificity
RN 39379-15-2 (Neurotensin); 56-41-7 (Alanine)
CN 0 (Fluorescent Dyes); EC 3.4.21 (Serine Endopeptidases); EC 3.4.21.26 (prolyl oligopeptidase); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.11 (Neprilysin); EC 3.4.24.15 (thimet oligopeptidase); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 13 OF 48 MEDLINE
AN 2001220470 MEDLINE
DN 21181614 PubMed ID: 11284698
TI Substrate specificity characterization of recombinant metallo oligo-peptidases thimet oligopeptidase and **neurolysin**.
AU Oliveira V; Campos M; Melo R L; Ferro E S; Camargo A C; Juliano M A; Juliano L
CS Department of Biophysics, Escola Paulista de Medicina, Universidade Federal de Sao Paulo, Rua Tres de Maio, 100 Sao Paulo - SP - 04044-020, Brazil.
SO BIOCHEMISTRY, (2001 Apr 10) 40 (14) 4417-25.
Journal code: 0370623. ISSN: 0006-2960.

CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200106
ED Entered STN: 20010702
Last Updated on STN: 20010702
Entered Medline: 20010628
AB We report a systematic and detailed analysis of recombinant **neurolysin** (EC 3.4.24.16) specificity in parallel with thimet oligopeptidase (TOP, EC 3.4.24.15) using Bk sequence and its C- and N-terminal extensions as in human kininogen as motif for synthesis of internally quenched fluorescent substrates. The influence of the substrate size was investigated, and the longest peptide susceptible to TOP and **neurolysin** contains 17 amino acids. The specificities of both oligopeptidases to substrate sites P(4) to P(3)' were also characterized in great detail using seven series of peptides based on Abz-GFSPFRQ-EDDnp taken as reference substrate. Most of the peptides were hydrolyzed at the bond corresponding to P(4)-F(5) in the reference substrate and some of them were hydrolyzed at this bond or at F(2)-S(3) bond. No restricted specificity was found for P(1)' as found in thermolysin as well for P(1) substrate position, however the modifications at this position (P(1)) showed to have large influence on the catalytic constant and the best substrates for TOP contained at P(1), Phe, Ala, or Arg and for **neurolysin** Asn or Arg. Some amino acid residues have large influence on the K(m) constants independently of its position. On the basis of these results, we are hypothesizing that some amino acids of the substrates can bind to different sub-sites of the enzyme fitting P-F or F-S bond, which requires rapid interchange for the different forms of interaction and convenient conformations of the substrate in order to expose and fit the cleavage bonds in correct position for an efficient hydrolysis. Finally, this plasticity of interaction with the substrates can be an essential property for a class of cytosolic oligopeptidases that are candidates to participate in the selection of the peptides to be presented by the MHC class I.
CT Check Tags: Animal; Comparative Study; Human; Male; Support, Non-U.S. Gov't
Amino Acid Sequence
Anthranilic Acids: ME, metabolism
Chromogenic Compounds: ME, metabolism
Ethylenediamines: ME, metabolism
Hydrolysis
Kinetics
Kininogens: ME, metabolism
Metalloendopeptidases: CH, chemistry
*Metalloendopeptidases: ME, metabolism
Molecular Sequence Data
Oligopeptides: CS, chemical synthesis
Oligopeptides: ME, metabolism
Rats
Recombinant Proteins: CH, chemistry
*Recombinant Proteins: ME, metabolism
Spectrum Analysis, Mass
Substrate Specificity
Swine
RN 28767-75-1 (N-(2,4-dinitrophenyl)ethylenediamine)
CN 0 (Anthranilic Acids); 0 (Chromogenic Compounds); 0 (Ethylenediamines); 0

(Kininogens); 0 (Oligopeptides); 0 (Recombinant Proteins); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.15 (thimet oligopeptidase); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 14 OF 48 MEDLINE
AN 2001092608 MEDLINE
DN 20545951 PubMed ID: 11092934
TI Crystallization and preliminary analysis of **neurolysin**.
AU Lian W; Chen G; Wu D; Brown C K; Madauss K; Hersh L B; Rodgers D W
CS Department of Biochemistry and Center for Structural Biology, University of Kentucky, Lexington, Kentucky 40536, USA.
SO ACTA CRYSTALLOGRAPHICA. SECTION D: BIOLOGICAL CRYSTALLOGRAPHY, (2000 Dec) 56 Pt 12 1644-6.
Journal code: 9305878. ISSN: 0907-4449.
CY Denmark
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200101
ED Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010125
AB Neuropeptidases inactivate or modify the activity of peptide neurotransmitters and neurohormones. The neuropeptidase **neurolysin** acts only on short peptides and accepts a variety of cleavage-site sequences. Structures of the enzyme and enzyme-substrate complexes will help to determine the mechanisms of substrate selectivity used by this enzyme. Crystals of recombinant **neurolysin** have been grown in the orthorhombic space group P2(1)2(1)2, with unit-cell parameters a = 157.8, b = 88.0, c = 58.4 A. Data have been collected to 2.3 A at 110 K with observed diffraction to 1.8 A. Circular dichroism measurements suggest that the enzyme is primarily alpha-helical, with little beta-strand secondary structure. Sequence-based secondary-structure prediction supports this conclusion.
CT Check Tags: Animal; Support, U.S. Gov't, Non-P.H.S.
Circular Dichroism
Crystallization
Escherichia coli
*Metalloendopeptidases: CH, chemistry
Metalloendopeptidases: GE, genetics
Metalloendopeptidases: ME, metabolism
Protein Structure, Secondary
Rats
Recombinant Proteins: CH, chemistry
Recombinant Proteins: ME, metabolism
Substrate Specificity
X-Ray Diffraction
CN 0 (Recombinant Proteins); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 15 OF 48 MEDLINE
AN 2001075880 MEDLINE
DN 20368033 PubMed ID: 10912905
TI Soluble neutral metallopeptidases: physiological regulators of peptide action.
AU Shrimpton C N; Smith A I
CS Baker Medical Research Institute, Melbourne, Victoria, Australia.
SO JOURNAL OF PEPTIDE SCIENCE, (2000 Jun) 6 (6) 251-63. Ref: 88
Journal code: 9506309. ISSN: 1075-2617.
CY ENGLAND: United Kingdom

DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Priority Journals
EM 200101
ED Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010111
AB Classically, the pre- and post-secretory processing of peptide signals appears to be mediated primarily by subtilisin-like peptidases in secretory vesicles and/or membrane-associated neutral endopeptidases in the extracellular environment. This article presents both biochemical and physiological evidence to support a role for soluble neutral metallopeptidases in the mediation of cell-to-cell communication by the selective generation and termination of peptide signals. These soluble peptidases have been implicated in the normal and disease-state processing of peptides involved in neurological, endocrine and cardiovascular functions. In this context, specific inhibitors of these enzymes could selectively modulate peptide levels and thus have considerable therapeutic potential. The aim of this review is to discuss the design and development of specific inhibitors of soluble neutral metallopeptidases that have been instrumental in identifying the roles of these enzymes. It will also review the evidence and present a case for the involvement of soluble neutral metallopeptidases in the regulation of peptide signalling in both central nervous system (CNS) and peripheral tissues.
CT Check Tags: Animal; Human; Support, Non-U.S. Gov't
Amino Acid Sequence
Cell Communication
Central Nervous System
Metalloendopeptidases: AI, antagonists & inhibitors
*Metalloendopeptidases: CH, chemistry
*Metalloendopeptidases: PH, physiology
Models, Chemical
Molecular Sequence Data
Peptide Hydrolases: ME, metabolism
*Peptides: ME, metabolism
Protein Processing, Post-Translational
Rats
Sequence Homology, Amino Acid
Subtilisin: ME, metabolism
CN 0 (Peptides); EC 3.4 (Peptide Hydrolases); EC 3.4.21.62 (Subtilisin); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.15 (thimet oligopeptidase); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 16 OF 48 MEDLINE
AN 2000464050 MEDLINE
DN 20469095 PubMed ID: 11016880
TI Bradykinin analogues with beta-amino acid substitutions reveal subtle differences in substrate specificity between the endopeptidases EC 3.4.24.15 and EC 3.4.24.16.
AU Lew R A; Boulos E; Stewart K M; Perlmutter P; Harte M F; Bond S; Aguilar M I; Smith A I
CS Baker Medical Research Institute, Melbourne, Victoria, Australia.
SO JOURNAL OF PEPTIDE SCIENCE, (2000 Sep) 6 (9) 440-5.

Journal code: 9506309. ISSN: 1075-2617.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200101
ED Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010125
AB The closely related zinc metalloendopeptidases EC 3.4.24.15 (EP24.15) and EC 3.4.24.16 (EP24.16) cleave many common substrates, including bradykinin (BK). As such, there are few substrate-based inhibitors which are sufficiently selective to distinguish their activities. We have used BK analogues with either alanine or beta-amino acid (containing an additional carbon within the peptide backbone) substitutions to elucidate subtle differences in substrate specificity between the enzymes. The cleavage of the analogues by recombinant EP24.15 and EP24.16 was assessed, as well as their ability to inhibit the two enzymes. Alanine-substituted analogues were generally better substrates than BK itself, although differences between the peptidases were observed. Similarly, substitution of the four N-terminal residues with beta-glycine enhanced cleavage in some cases, but not others. beta-Glycine substitution at or near the scissile bond (Phe5-Ser6) completely prevented cleavage by either enzyme: interestingly, these analogues still acted as inhibitors, although with very different affinities for the two enzymes. Also of interest, beta-Gly8-BK was neither a substrate nor an inhibitor of EP24.15, yet could still interact with EP24.16. Finally, while both enzymes could be similarly inhibited by the D-stereoisomer of beta-C3-Phe5-BK (IC50 approximately 20 microM, compared to 8 microM for BK), EP24.16 was relatively insensitive to the L-isomer (IC50 12 approximately microM for EP24.15, >40 microM for EP24.16). These studies indicate subtle differences in substrate specificity between EP24.15 and EP24.16, and suggest that beta-amino acid analogues may be useful as templates for the design of selective inhibitors.
CT Check Tags: Animal; Comparative Study; Support, Non-U.S. Gov't
Alanine: CH, chemistry
Amino Acid Substitution
Bradykinin: AA, analogs & derivatives
*Bradykinin: ME, metabolism
Bradykinin: PD, pharmacology
Dose-Response Relationship, Drug
Glycine: CH, chemistry
Hydrolysis
Kinetics
Metalloendopeptidases: AI, antagonists & inhibitors
*Metalloendopeptidases: ME, metabolism
Peptide Fragments: ME, metabolism
Rats
Substrate Specificity
RN 56-40-6 (Glycine); 56-41-7 (Alanine); 58-82-2 (Bradykinin)
CN 0 (Peptide Fragments); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.15 (thimet oligopeptidase); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 17 OF 48 MEDLINE
AN 2000107466 MEDLINE
DN 20107466 PubMed ID: 10642854
TI Differential subcellular distribution of **neurolysin** (EC 3.4.24.16) and thimet oligopeptidase (EC 3.4.24.15) in the rat brain.
AU Massarelli E E; Casatti C A; Kato A; Camargo A C; Bauer J A; Glucksman M J; Roberts J L; Hirose S; Ferro E S
CS Department of Histology, Biomedical Sciences Institute, USP, SP, Brazil.
SO BRAIN RESEARCH, (1999 Dec 18) 851 (1-2) 261-5.
Journal code: 0045503. ISSN: 0006-8993.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200002
ED Entered STN: 20000309
Last Updated on STN: 20000309
Entered Medline: 20000224
AB Immunohistochemistry was used to analyze the rat brain distribution of thimet oligopeptidase and **neurolysin**. Both enzymes appear ubiquitously distributed within the entire rat brain. However, neuronal perikarya and processes stained for **neurolysin**, while intense nuclear labeling was only observed for thimet oligopeptidase. These data suggest that **neurolysin** and thimet oligopeptidase, endopeptidases sharing several functional and structural similarities, are present in distinctive intracellular compartments in neuronal cells.
CT Check Tags: Animal; Male; Support, Non-U.S. Gov't
*Brain Chemistry
*Metalloendopeptidases: AN, analysis
*Neurons: CH, chemistry
Rats
Rats, Wistar
CN EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.15 (thimet oligopeptidase); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 18 OF 48 MEDLINE
AN 1999249194 MEDLINE
DN 99249194 PubMed ID: 10235115
TI Confocal microscopy reveals thimet oligopeptidase (EC 3.4.24.15) and **neurolysin** (EC 3.4.24.16) in the classical secretory pathway.
AU Garrido P A; Vandenbulcke F; Ramjaun A R; Vincent B; Checler F; Ferro E; Beaudet A
CS Department of Histology and Embryology, Biomedical Science Institute, University of Sao Paulo, Brazil.
SO DNA AND CELL BIOLOGY, (1999 Apr) 18 (4) 323-31.
Journal code: 9004522. ISSN: 1044-5498.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199905
ED Entered STN: 19990614
Last Updated on STN: 20000303
Entered Medline: 19990528
AB Thimet oligopeptidase (EC 3.4.24.15; EP24.15) and **neurolysin** (EC 3.4.24.16; EP24.16) are closely related enzymes involved in the metabolic inactivation of bioactive peptides. Both of these enzymes were previously shown to be secreted from a variety of cell types, although their primary

sequence lacks a signal peptide. To investigate the mechanisms responsible for this secretion, we examined by confocal microscopy the subcellular localization of these two enzymes in the neuroendocrine cell line AtT20. Both EP24.15 and EP24.16 were found by immunohistochemistry to be abundantly expressed in AtT20 cells. Western blotting experiments confirmed that the immunoreactivity detected in the soma of these cells corresponded to previously cloned isoforms of the enzymes. At the subcellular level, both enzymes colocalized extensively with the integral trans-Golgi network protein, syntaxin-6, in the juxtanuclear region. In addition, both EP24.15 and EP24.16 were found within small vesicular organelles distributed throughout the cell body. Some, but not all, of these organelles also stained positively for ACTH. These results demonstrate that both EP24.15 and EP24.16 are present within the classical secretory pathway. Their colocalization with ACTH further suggests that they may be targeted to the regulated secretory pathway, even in the absence of a signal peptide.

CT Check Tags: Animal; Support, Non-U.S. Gov't
Blotting, Western
*Metalloendopeptidases: ME, metabolism
*Microscopy, Confocal: MT, methods
Rabbits

CN EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.15 (thimet oligopeptidase); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 19 OF 48 MEDLINE

AN 1999195469 MEDLINE

DN 99195469 PubMed ID: 10095765

TI Purification and characterization of a detergent-requiring membrane-bound metalloendopeptidase from porcine brain.

AU Jeohn G H; Matsuzaki H; Takahashi K

CS Laboratory of Molecular Biochemistry, School of Life Science, Tokyo University of Pharmacy and Life Science, Japan.

SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (1999 Mar) 260 (2) 318-24. Journal code: 0107600. ISSN: 0014-2956.

CY GERMANY: Germany, Federal Republic of

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 199904

ED Entered STN: 19990511
Last Updated on STN: 20000303
Entered Medline: 19990426

AB A detergent-requiring metalloendopeptidase cleaving a progastrin-C-terminal peptide (progastrin-(88-101)) mainly at the Arg95-Gly96 bond was solubilized from porcine cerebral vesicular membranes and purified to homogeneity as examined by PAGE. The purified enzyme had a molecular mass of approximately 76 kDa as estimated by both SDS/PAGE and Sephacryl S-300 gel filtration. It hydrolyzed progastrin-(88-101) peptide, BAM-12P, and bradykinin fairly specifically, and more efficiently than various other neuropeptides and related oligopeptides examined as substrates. It was inactive in the absence of detergents, and required certain detergents such as Triton X-100 or Lubrol PX for activity. Its optimum pH was about 6.5 and was strongly inhibited by metal-chelating agents such as EDTA, EGTA, and o-phenanthroline. It was extremely sensitive to EDTA and was completely inhibited even by 0.3 microM EDTA; the activity was fully restored by addition of a 10-fold higher concentration of Zn2+, CO2+, or Mn2+ ions over EDTA. On the other hand, dynorphin A-(1-13) peptide, a strong inhibitor of **neurolysin**, failed to inhibit the enzyme.

The various characteristics indicated that the present enzyme is a unique membrane-bound metalloendopeptidase.

CT Check Tags: Animal
Amino Acid Sequence
*Brain: EN, enzymology
Centrifugation, Density Gradient
Cobalt: ME, metabolism
*Coumarins: ME, metabolism
Detergents
Dynorphins: PD, pharmacology
Electrophoresis, Polyacrylamide Gel
Intracellular Membranes: EN, enzymology
Manganese: ME, metabolism
*Metalloendopeptidases: IP, isolation & purification
Metalloendopeptidases: ME, metabolism
Molecular Sequence Data
Molecular Weight
*Oligopeptides: ME, metabolism
Protease Inhibitors: PD, pharmacology
Solubility
Substrate Specificity
Swine
Zinc: ME, metabolism

RN 7439-96-5 (Manganese); 7440-48-4 (Cobalt); 7440-66-6 (Zinc); 74913-18-1 (Dynorphins)

CN 0 (Boc-Gly-Arg-Arg-MCA); 0 (Coumarins); 0 (Detergents); 0 (Oligopeptides); 0 (Protease Inhibitors); EC 3.4.24 (Metalloendopeptidases)

L6 ANSWER 20 OF 48 MEDLINE

AN 1998407880 MEDLINE

DN 98407880 PubMed ID: 9735321

TI Neuropeptide specificity and inhibition of recombinant isoforms of the endopeptidase 3.4.24.16 family: comparison with the related recombinant endopeptidase 3.4.24.15.

AU Rioli V; Kato A; Portaro F C; Cury G K; te Kaat K; Vincent B; Checler F; Camargo A C; Glucksman M J; Roberts J L; Hirose S; Ferro E S

CS Biomedical Science Institute, University of Sao Paulo, 05508-900, Brazil.

NC DK45493 (NIDDK)

SO BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1998 Sep 8) 250 (1) 5-11.
Journal code: 0372516. ISSN: 0006-291X.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 199810

ED Entered STN: 19981020
Last Updated on STN: 20000303
Entered Medline: 19981007

AB Endopeptidase EC 3.4.24.16 (EP24.16c, **neurolysin**) and thimet oligopeptidase EC 3.4.24.15 are close related members of a large family of metalloproteases. Besides their cytosolic and membrane bound form, endopeptidase EC 3.4.24.16 appears to be present in the inner membrane of the mitochondria (EP24.16m). We have overexpressed two porcine EP24.16 isoforms in E. coli and purified the recombinant proteins to homogeneity. We show here that these peptidases hydrolyse a series of neuropeptides with similar rates and at sites reminiscent of those elicited by

classically purified human brain EP24.16c. All neuropeptides, except neurotensin, were similarly cleaved by recombinant endopeptidase 3.4.24.15 (EP24.15, thimet oligopeptidase), another zinc-containing metalloenzyme structurally related to EP24.16. These two EP24.16 isoforms were drastically inhibited by Pro-Ile and dithiothreitol and remained unaffected by a specific carboalkyl inhibitor (CFP-AAY-pAb) directed toward the related EP24.15. The present purification procedure of EP24.16 should allow to establish, by mutagenesis analysis, the mechanistic properties of the enzyme.

CT Check Tags: Animal; Comparative Study; Human; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.
Amino Acid Sequence
Base Sequence
Cytosol: EN, enzymology
DNA, Complementary
Enzyme Activation
Hydrolysis
Isoenzymes: AI, antagonists & inhibitors
Isoenzymes: GE, genetics
*Isoenzymes: ME, metabolism
Metalloendopeptidases: AI, antagonists & inhibitors
Metalloendopeptidases: GE, genetics
*Metalloendopeptidases: ME, metabolism
Mitochondria: EN, enzymology
Molecular Sequence Data
*Neuropeptides: ME, metabolism
Recombinant Proteins: AI, antagonists & inhibitors
Recombinant Proteins: GE, genetics
Recombinant Proteins: ME, metabolism
Substrate Specificity
Swine

CN 0 (DNA, Complementary); 0 (Isoenzymes); 0 (Neuropeptides); 0 (Recombinant Proteins); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.15 (thimet oligopeptidase); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 21 OF 48 MEDLINE
AN 97401381 MEDLINE
DN 97401381 PubMed ID: 9257187
TI Characterization and localization of mitochondrial oligopeptidase (MOP) (EC 3.4.24.16) activity in the human cervical adenocarcinoma cell line HeLa.
AU Krause D R; Piva T J; Brown S B; Ellem K A
CS QCF Cancer Research Unit, Queensland Institute of Medical Research, Post Office Royal Brisbane Hospital, Australia.
SO JOURNAL OF CELLULAR BIOCHEMISTRY, (1997 Sep 1) 66 (3) 297-308.
Journal code: 8205768. ISSN: 0730-2312.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199709
ED Entered STN: 19971008
Last Updated on STN: 20000303
Entered Medline: 19970925
AB In this study we describe the partial purification and characterization of the HeLa cell oligopeptidase M or endopeptidase 3.4.24.16. The HeLa

enzyme was isolated initially by its ability to hydrolyse a nonapeptide substrate (P9) which was cognate to the N-terminal cleavage site of preproTGF alpha. The enzyme was shown to be a metalloprotease as it was inhibited by Zn(2+)-chelating agents and DTT, and had an approximate molecular weight of 55-63 kD determined by gel filtration. Neurotensin, dynorphin A1-17 and GnRH1-9 were rapidly degraded by the enzyme while GnRH1-10 and somatostatin were not. Neurotensin was cleaved at the Pro10-Tyr11 bond, leading to the formation of neurotensin (1-10) and neurotensin (11-13). The K(m) for neurotensin cleavage was 7 microM and the Ki for the specific 24.16 dipeptide inhibitor (Pro-ile) was 140 microM
which were similar to those observed from the human brain enzyme [Vincent et al. (1996): Brain Res 709:51-58]. Through the use of specific antibodies, the purified HeLa enzyme was shown to be oligopeptidase M. This enzyme and its closely related family member thimet oligopeptidase were shown to co-elute during the isolation procedure but were finally separated using a MonoQ column. Oligopeptidase M is located mainly in mitochondria though it was detected on the plasma membrane in an inactive form. The results obtained demonstrate the first recorded instance of this enzyme in human tissue cultured cells, and raise the issue of its function therein.

CT Check Tags: Human; Support, Non-U.S. Gov't
Cell Membrane: EN, enzymology
Edetic Acid: PD, pharmacology
*Hela Cells: EN, enzymology
Isoenzymes
Metalloendopeptidases: AI, antagonists & inhibitors
*Metalloendopeptidases: IP, isolation & purification
*Metalloendopeptidases: ME, metabolism
*Mitochondria: EN, enzymology
Plasma: EN, enzymology
Protease Inhibitors: PD, pharmacology
RN 60-00-4 (Edetic Acid)
CN 0 (Isoenzymes); 0 (Protease Inhibitors); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 22 OF 48 MEDLINE
AN 97351855 MEDLINE
DN 97351855 PubMed ID: 9208137
TI Effect of a novel selective and potent phosphinic peptide inhibitor of endopeptidase 3.4.24.16 on neurotensin-induced analgesia and neuronal inactivation.
AU Vincent B; Jiracek J; Noble F; Loog M; Roques B; Dive V; Vincent J P; Checler F
CS IPMC du CNRS, UPR411, Valbonne, France.
SO BRITISH JOURNAL OF PHARMACOLOGY, (1997 Jun) 121 (4) 705-10.
Journal code: 7502536. ISSN: 0007-1188.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199709
ED Entered STN: 19971008
Last Updated on STN: 20000303
Entered Medline: 19970925
AB 1. We have examined a series of novel phosphinic peptides as putative potent and selective inhibitors of endopeptidase 3.4.24.16. 2. The most selective inhibitor, Pro-Phe-psi(PO2CH2)-Leu-Pro-NH2 displayed a Ki value of 12 nM towards endopeptidase 3.4.24.16 and was 5540 fold less potent on its related peptidase endopeptidase 3.4.24.15. Furthermore, this

inhibitor was 12.5 less potent on angiotensin-converting enzyme and was unable to block endopeptidase 3.4.24.11, aminopeptidases B and M, dipeptidylaminopeptidase IV and proline endopeptidase. 3. The effect of Pro-Phe-psi(PO2CH2)-Leu-Pro-NH2, in vitro and in vivo, on neurotensin metabolism in the central nervous system was examined. 4. Pro-Phe-psi(PO2CHH2)-Leu-Pro-NH2 dose-dependently inhibited the formation of neurotensin 1-10 and concomittantly protected neurotensin from degradation by primary cultured neurones from mouse embryos. 5. Intracerebroventricular administration of Pro-Phe-psi(PO2CH2)-Leu-Pro-NH2 significantly potentiated the neurotensin-induced antinociception of mice in the hot plate test. 6. Altogether, our study has established Pro-Phe-psi(PO2CH2)-Leu-Pro-NH2 as a fully selective and highly potent inhibitor of endopeptidase 3.4.24.16 and demonstrates, for the first time, the contribution of this enzyme in the central metabolism of neurotensin.

CT Check Tags: Animal; Male; Support, Non-U.S. Gov't
Analgesia
*Metalloendopeptidases: AI, antagonists & inhibitors
Mice
*Neurons: DE, drug effects
*Neurotensin: ME, metabolism
*Oligopeptides: PD, pharmacology
Peptidyl-Dipeptidase A: ME, metabolism
*Phosphines: PD, pharmacology
Rats

RN 39379-15-2 (Neurotensin)

CN 0 (Oligopeptides); 0 (Phosphines); 0 (prolyl-phenylalanyl-psi(PO2CH2)-leucyl-prolinamide); EC 3.4.15.1 (Peptidyl-Dipeptidase A); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 23 OF 48 MEDLINE

AN 97326108 MEDLINE

DN 97326108 PubMed ID: 9182559

TI Targeting of endopeptidase 24.16 to different subcellular compartments by alternative promoter usage.

AU Kato A; Sugiura N; Saruta Y; Hosoiri T; Yasue H; Hirose S

CS Department of Biological Sciences, Tokyo Institute of Technology, 4259 Nagatsuta-cho, Midori-ku, Yokohama 226, Japan.

SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1997 Jun 13) 272 (24) 15313-22.
Journal code: 2985121R. ISSN: 0021-9258.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

OS GENBANK-AB000170; GENBANK-AB000171; GENBANK-AB000172; GENBANK-AB000173; GENBANK-AB000174; GENBANK-AB000175; GENBANK-AB000411; GENBANK-AB000412; GENBANK-AB000413; GENBANK-AB000414; GENBANK-AB000415; GENBANK-AB000416; GENBANK-AB000417; GENBANK-AB000418; GENBANK-AB000419; GENBANK-AB000420; GENBANK-AB000421; GENBANK-AB000422; GENBANK-AB000423; GENBANK-AB000424; GENBANK-AB000425; GENBANK-AB000426; GENBANK-AB000427; GENBANK-AB000428; GENBANK-AB000429; GENBANK-AB000430; GENBANK-AB000431

EM 199707

ED Entered STN: 19970724
Last Updated on STN: 20000303
Entered Medline: 19970714

AB Endopeptidase 24.16 or mitochondrial oligopeptidase, abbreviated here as EP 24.16 (MOP), is a thiol- and metal-dependent oligopeptidase that is found in multiple intracellular compartments in mammalian cells. From an analysis of the corresponding gene, we found that the distribution of the enzyme to appropriate subcellular locations is achieved by the use of

alternative sites for the initiation of transcription. The pig EP 24.16 (MOP) gene spans over 100 kilobases and is organized into 16 exons. The core protein sequence is encoded by exons 5-16 which match perfectly with exons 2-13 of the gene for endopeptidase 24.15, another member of the thimet oligopeptidase family. These two sets of 11 exons share the same splice sites, suggesting a common ancestor. Multiple species of mRNA for EP 24.16 (MOP) were detected by the 5'-rapid amplification of cDNA ends and they were shown to have been generated from a single gene by alternative choices of sites for the initiation of transcription and splicing. Two types of transcript were prepared, corresponding to transcription from distal and proximal sites. Their expression in vitro in COS-1 cells indicated that they encoded two isoforms (long and short) which differed only at their amino termini: the long form contained a cleavable mitochondrial targeting sequence and was directed to mitochondria; the short form, lacking such a signal sequence, remained in the cytosol. The complex structure of the EP 24.16 (MOP) gene thus allows, by alternative promoter usage, a fine transcriptional regulation of coordinate expression, in the different subcellular compartments, of the two isoforms arising from a single gene.

CT Check Tags: Animal; Support, Non-U.S. Gov't
Amino Acid Sequence
Base Sequence
DNA, Complementary
Exons
Metalloendopeptidases: GE, genetics
*Metalloendopeptidases: ME, metabolism
Molecular Sequence Data
*Promoter Regions (Genetics)
Protein Binding
RNA, Messenger: GE, genetics
Sequence Homology, Nucleic Acid
*Subcellular Fractions: EN, enzymology
Swine
Transcription Factors: ME, metabolism
Transcription, Genetic

CN 0 (DNA, Complementary); 0 (RNA, Messenger); 0 (Transcription Factors); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.15 (thimet oligopeptidase); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 24 OF 48 MEDLINE
AN 97156686 MEDLINE
DN 97156686 PubMed ID: 9003076
TI Stably transfected human cells overexpressing rat brain endopeptidase 3.4.24.16: biochemical characterization of the activity and expression of soluble and membrane-associated counterparts.
AU Vincent B; Dauch P; Vincent J P; Checler F
CS IPMC du CNRS, UPR 411, Valbonne, France.
SO JOURNAL OF NEUROCHEMISTRY, (1997 Feb) 68 (2) 837-45.
Journal code: 2985190R. ISSN: 0022-3042.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199702
ED Entered STN: 19970306
Last Updated on STN: 20000303
Entered Medline: 19970221
AB We recently cloned endopeptidase-24.16 (**neurolysin**; EC 3.4.24.16), a neurotensin-degrading peptidase likely involved in the physiological termination of the neurotensinergic signal in the central

nervous system and in the gastrointestinal tract. We stably transfected human kidney cells with the pcDNA3-lambda 7aB1 construction bearing the whole open reading frame encoding the rat brain peptidase. Transfectants displayed endopeptidase-24.16 immunoreactivity and exhibited QFS- and neurotensin-hydrolyzing activities, the biochemical and specificity properties of which fully matched those observed with the purified murine enzyme. Cryoprotection experiments and substrate degradation by intact plated cells indicated that transfectants exhibited a membrane-associated form of endopeptidase-24.16, the catalytic site of which clearly faced the
extracellular domain. Transfected cells were unable to secrete the enzyme. Overall, our experiments indicate that we have obtained stably transfectant cells that overexpress an enzymatic activity displaying biochemical properties identical to those of purified endopeptidase-24.16.
The membrane-associated counterpart and lack of secretion of the enzyme were clearly reminiscent of what was observed with pure cultured neurons, but not with astrocytes. Therefore, the transfected cell model described here could prove useful for establishing, by a mutagenesis approach, the structural elements responsible for the "neuronal" phenotype exhibited by the enzyme in transfected cells.

CT Check Tags: Animal; Human; Support, Non-U.S. Gov't
Blotting, Western
Brain: EN, enzymology
Cell Line: PH, physiology
Digitonin: PD, pharmacology
Enzyme Activation: DE, drug effects
Gene Expression Regulation, Enzymologic: PH, physiology
Immunohistochemistry
Indicators and Reagents: PD, pharmacology
Kidney: CY, cytology
Membrane Proteins: ME, metabolism
*Metalloendopeptidases: CH, chemistry
*Metalloendopeptidases: ME, metabolism
Metalloendopeptidases: SE, secretion
Protein Binding: PH, physiology
Rats
Sodium Bicarbonate: PD, pharmacology
Sodium Chloride: PD, pharmacology
Solubility
*Transfection

RN 11024-24-1 (Digitonin); 144-55-8 (Sodium Bicarbonate); 7647-14-5 (Sodium Chloride)

CN 0 (Indicators and Reagents); 0 (Membrane Proteins); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 25 OF 48 MEDLINE

AN 97026353 MEDLINE

DN 97026353 PubMed ID: 8872532

TI New hydroxamate inhibitors of neurotensin-degrading enzymes. Synthesis and
enzyme active-site recognition.

AU Bourdel E; Doulut S; Jarretou G; Labbe-Jullie C; Fehrentz J A; Doumbia O; Kitabgi P; Martinez J

CS Laboratory of Aminoacids, Peptides and Proteins, LAPP, ESA CNRS 5075, Universities of Montpellier I and Montpellier II, Faculty of Pharmacy, France.

SO INTERNATIONAL JOURNAL OF PEPTIDE AND PROTEIN RESEARCH, (1996 Aug) 48 (2) 148-55.
Journal code: 0330420. ISSN: 0367-8377.

CY Denmark
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199701
ED Entered STN: 19970219
Last Updated on STN: 20000303
Entered Medline: 19970127
AB Selective and mixed inhibitors of the three zinc metallopeptidases that degrade neurotensin (NT), e.g. endopeptidase 24-16 (EC 3.4.24.16), endopeptidase 24-11 (EC 3.4.24.11 or neutral endopeptidase, NEP) and endopeptidase 24-15 (EC 3.4.24.15), and leucine-aminopeptidase (type IV-S), that degrades the NT-related peptides, Neuromedin N (NN), are of great interest. On the structural basis of compound JMV 390-1 (N-[3-[(hydroxyamino)carbonyl]-1-oxo-2(R)-benzylpropyl]-L-isoleucyl-L-leucine), which was a full inhibitor of the major NT degrading
enzymes, several hydroxamate inhibitors corresponding to the general formula HONHCO-CH2-CH(CH2-C6H5)CO-X-Y-OH (with X-Y = dipeptide) have been synthesized. Compound 7a (X-Y = Ile-Ala) was nearly 40-times more potent in inhibiting EC 24-16 than NEP and more than 800-times more potent than EC 24-15, with an IC50 (12 nM) almost equivalent to that of compound JMV 390-1. Therefore, this compound is an interesting selective inhibitor of EC 24-16, and should be an interesting probe to explore the physiological involvement of EC 24-16 in the metabolism of neurotensin.
CT Check Tags: Animal
Binding Sites
Brain: EN, enzymology
Enzyme Inhibitors: CS, chemical synthesis
*Enzyme Inhibitors: PD, pharmacology
Hydrolysis
Hydroxamic Acids: CS, chemical synthesis
*Hydroxamic Acids: PD, pharmacology
*Metalloendopeptidases: ME, metabolism
*Neprilysin: ME, metabolism
*Neurotensin: ME, metabolism
Rats
RN 39379-15-2 (Neurotensin)
CN 0 (Enzyme Inhibitors); 0 (Hydroxamic Acids); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.11 (Neprilysin); EC 3.4.24.15 (thimet oligopeptidase); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 26 OF 48 MEDLINE
AN 97023196 MEDLINE
DN 97023196 PubMed ID: 8869556
TI Purification and characterization of human endopeptidase 3.4.24.16. Comparison with the porcine counterpart indicates a unique cleavage site on neurotensin.
AU Vincent B; Vincent J P; Checler F
CS I.P.M.C.-CNRS, Valbonne, France.
SO BRAIN RESEARCH, (1996 Feb 12) 709 (1) 51-8.
Journal code: 0045503. ISSN: 0006-8993.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199701
ED Entered STN: 19970219
Last Updated on STN: 20000303
Entered Medline: 19970129

AB We have purified and characterized human brain endopeptidase 3.4.24.16. The enzyme behaved as a 72 kDa protein and belonged to the metalloprotease family. Human endopeptidase 3.4.24.16 cleaved neurotensin at a unique site at the Pro10-Tyr11 bond, leading to the formation of neurotensin(1-10) and neurotensin(11-13). The kinetic parameters displayed by human endopeptidase 3.4.24.16 towards a series of natural neuropeptides indicated that bradykinin was the most efficiently proteolysed. Angiotensin I, dynorphins 1-8 and 1-9 and substance P also behaved as good substrates while neuromedin N, angiotensin II, leucine and methionine enkephalin and neurokinin A resisted degradation by human endopeptidase 3.4.24.16. We have purified the porcine counterpart of endopeptidase 3.4.24.16 and compared its ability to cleave neurotensin with that of the enzyme from human origin. It appeared that, besides a major production of neurotensin(1-10), an additional formation of neurotensin(1-8) was observed with the pig enzyme, suggesting a cleavage of neurotensin not only at the Pro10-Tyr11 bond but also at the Arg8-Arg9 peptidyl bond. The latter cleavage appeared reminiscent of endopeptidase 3.4.24.15 since this peptidase was reported to cleave neurotensin at the Arg8-Arg9 bond. Our study indicated that neurotensin(1-10) formation by porcine endopeptidase 3.4.24.16 could be potently blocked with the selective endopeptidase 3.4.24.16 dipeptide inhibitor Pro-Ile without interfering with neurotensin(1-8) formation. By contrast, the formation of the latter product was highly potentiated by dithiothreitol and inhibited by the endopeptidase 3.4.24.15 inhibitor Cpp-Ala-Ala-Tyr-pAB, two effects that were not observed for neurotensin(1-10) production. Altogether, our results indicate that porcine endopeptidase 3.4.24.16 cleaves neurotensin at a unique site, leading to the formation of neurotensin(1-10) and that the production of neurotensin(1-8) is due to contaminating endopeptidase 3.4.24.15.

CT Check Tags: Animal; Comparative Study; Human; Support, Non-U.S. Gov't
Brain: ME, metabolism
Chromatography
Chromatography, High Pressure Liquid
Fluorometry
Metalloendopeptidases: CH, chemistry
*Metalloendopeptidases: IP, isolation & purification
*Metalloendopeptidases: ME, metabolism
*Neurotensin: ME, metabolism
Peptide Fragments: ME, metabolism
Rats
*Swine: ME, metabolism

RN 39379-15-2 (Neurotensin); 63524-00-5 (neurotensin (1-10)); 80887-44-1 (neurotensin (1-8))

CN 0 (Peptide Fragments); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.15 (thimet oligopeptidase); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 27 OF 48 MEDLINE

AN 96325082 MEDLINE

DN 96325082 PubMed ID: 8702656

TI Development of the first potent and selective inhibitor of the zinc endopeptidase **neurolysin** using a systematic approach based on combinatorial chemistry of phosphinic peptides.

AU Jiracek J; Yiotakis A; Vincent B; Checler F; Dive V

CS Commissariat a l'Energie Atomique, Departement d'Ingenierie et d'Etudes des Proteines, DSV, CE-Saclay 91191 Gif-sur-Yvette Cedex, France.

SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Aug 9) 271 (32) 19606-11. Journal code: 2985121R. ISSN: 0021-9258.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199609
ED Entered STN: 19960924
Last Updated on STN: 20000303
Entered Medline: 19960916
AB A new systematic approach, based on combinatorial chemistry of phosphinic peptides, is proposed for rapid development of highly potent and selective inhibitors of zinc metalloproteases. This strategy first evaluates the effects on the inhibitory potency and selectivity of the following parameters: 1) size of the phosphinic peptides, 2) position of the phosphinic bond in the sequence, and 3) the state (free or blocked) of the peptide extremities. After this selection step, the influence of the inhibitor sequence is analyzed in order to determine the identity of the residues that optimized both the potency and the selectivity. We demonstrate the efficiency of this novel approach in rapid identification of the first potent inhibitor of the mammalian zinc endopeptidase **neurolysin**(24-16), able to discriminate between this enzyme and the related zinc endopeptidase thimet oligopeptidase(24-15). The most potent and selective inhibitor developed in this study, Pro-LPhePsi(PO2CH2)Gly-Pro, displays a Ki value of 4 nM for 24-16 and is 2000 times less potent on 24-15. The specific recognition of such a free phosphinic tetrapeptide by 24-16, as well as the unique specificity of the 24-16 S2 and S2' subsites for proline, unveiled by this study, are discussed in terms of their possible significance for the function of this enzyme and its related zinc endopeptidase activities.
CT Check Tags: Animal
Amino Acid Sequence
*Metalloendopeptidases: AI, antagonists & inhibitors
Molecular Sequence Data
*Oligopeptides: CS, chemical synthesis
Oligopeptides: CH, chemistry
*Phosphinic Acids: CH, chemistry
*Protease Inhibitors: CS, chemical synthesis
Protease Inhibitors: CH, chemistry
Rats
CN 0 (Oligopeptides); 0 (Phosphinic Acids); 0 (Protease Inhibitors); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.15 (thimet oligopeptidase); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 28 OF 48 MEDLINE
AN 96322972 MEDLINE
DN 96322972 PubMed ID: 8756435
TI Distinct properties of neuronal and astrocytic endopeptidase 3.4.24.16: a study on differentiation, subcellular distribution, and secretion processes.
AU Vincent B; Beaudet A; Dauch P; Vincent J P; Checler F
CS Institut de Pharmacologie Moleculaire et Cellulaire, CNRS UPR 411, Valbonne, France.
SO JOURNAL OF NEUROSCIENCE, (1996 Aug 15) 16 (16) 5049-59.
Journal code: 8102140. ISSN: 0270-6474.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals

EM 199612
ED Entered STN: 19970128
Last Updated on STN: 20000303
Entered Medline: 19961213
AB Endopeptidase 3.4.24.16 belongs to the zinc-containing metalloprotease family and likely participates in the physiological inactivation of neurotensin. The peptidase displays distinct features in pure primary cultured neurons and astrocytes. Neuronal maturation leads to a decrease in the proportion of endopeptidase 3.4.24.16-bearing neurons and to a concomitant increase in endopeptidase 3.4.24.16 activity and mRNA content.

By contrast, there is no change with time in endopeptidase 3.4.24.16 activity or content in astrocytes. Primary cultured neurons exhibit both soluble and membrane-associated endopeptidase 3.4.24.16 activity. The latter behaves as an ectopeptidase on intact plated neurons and resists treatments with 0.2% digitonin and Na2CO3. Further evidence for an association of the enzyme with plasma membranes was provided by cryoprotection experiments and electron microscopic analysis. The membrane-associated form of endopeptidase 3.4.24.16 increased during neuronal differentiation and appears to be mainly responsible for the overall augmentation of endopeptidase 3.4.24.16 activity observed during neuronal maturation. Unlike neurons, astrocytes only contain soluble endopeptidase 3.4.24.16. Astrocytes secrete the enzyme through monensin, brefeldin A, and forskolin-independent mechanisms. This indicates that endopeptidase 3.4.24.16 is not released by classical regulated or constitutive secreting processes. However, secretion is blocked at 4 degrees C and by 8 bromo cAMP and is enhanced at 42 degrees C, two properties reminiscent of that of other secreted proteins lacking a classical signal peptide. By contrast, neurons appear unable to secrete endopeptidase 3.4.24.16.
CT Check Tags: Animal; Support, Non-U.S. Gov't
Astrocytes: CY, cytology
*Astrocytes: EN, enzymology
Cell Differentiation
Cells, Cultured
Immunologic Techniques
*Metalloendopeptidases: CH, chemistry
Metalloendopeptidases: GE, genetics
*Metalloendopeptidases: ME, metabolism
Mice: EM, embryology
Microscopy, Electron
Neurons: CY, cytology
*Neurons: EN, enzymology
RNA, Messenger: ME, metabolism
Subcellular Fractions: EN, enzymology
Tegmentum Mesencephali: ME, metabolism
Tegmentum Mesencephali: UL, ultrastructure
Tissue Distribution
CN 0 (RNA, Messenger); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 29 OF 48 MEDLINE
AN 96070836 MEDLINE
DN 96070836 PubMed ID: 7592986
TI Molecular cloning and expression of rat brain endopeptidase 3.4.24.16.
AU Dauch P; Vincent J P; Checler F
CS Institut de Pharmacologie Moleculaire et Cellulaire, Valbonne, France.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 Nov 10) 270 (45) 27266-71.
Journal code: 2985121R. ISSN: 0021-9258.
CY United States

DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199512
ED Entered STN: 19960124
Last Updated on STN: 20000303
Entered Medline: 19951226
AB We have isolated by immunological screening of a lambda ZAPII cDNA library
constructed from rat brain mRNAs a cDNA clone encoding endopeptidase 3.4.24.16. The longest open reading frame encodes a 704-amino acid protein with a theoretical molecular mass of 80,202 daltons and bears the consensus sequence of the zinc metalloprotease family. The sequence exhibits a 60.2% homology with those of another zinc metallopeptidase, endopeptidase 3.4.24.15. Northern blot analysis reveals two mRNA species of about 3 and 5 kilobases in rat brain, ileum, kidney, and testis. We have transiently transfected COS-7 cells with pcDNA3 containing the cloned
cDNA and established the overexpression of a 70-75-kDa immunoreactive protein. This protein hydrolyzes QFS, a quenched fluorimetric substrate of endopeptidase 3.4.24.16, and cleaves neurotensin at a single peptide bond, leading to the formation of neurotensin (1-10) and neurotensin (11-13). QFS and neurotensin hydrolysis are potently inhibited by the selective endopeptidase 3.4.24.16 dipeptide blocker Pro-Ile and by dithiothreitol, while the enzymatic activity remains unaffected by phosphoramidon and captopril, the specific inhibitors of endopeptidase 3.4.24.11 and angiotensin-converting enzyme, respectively. Altogether, these physicochemical, biochemical, and immunological properties unambiguously identify endopeptidase 3.4.24.16 as the protein encoded by the isolated cDNA clone.
CT Check Tags: Animal; Male; Support, Non-U.S. Gov't
Amino Acid Sequence
Base Sequence
*Brain: EN, enzymology
Cell Line
Cloning, Molecular
DNA, Complementary: GE, genetics
Gene Expression
Metalloendopeptidases: CH, chemistry
*Metalloendopeptidases: GE, genetics
Metalloendopeptidases: ME, metabolism
Molecular Sequence Data
Molecular Weight
Neurotensin: ME, metabolism
Oligopeptides: CH, chemistry
Oligopeptides: ME, metabolism
Open Reading Frames
RNA, Messenger: GE, genetics
RNA, Messenger: ME, metabolism
Rats
Substrate Specificity
Tissue Distribution
Transfection
RN 127376-94-7 (7-methoxycoumarin-3-carboxylyl-prolyl-leucyl-glycyl-prolyl-lysyl-2,4-dinitrophenyl); 39379-15-2 (Neurotensin)
CN 0 (DNA, Complementary); 0 (Oligopeptides); 0 (RNA, Messenger); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 30 OF 48 MEDLINE
AN 95405288 MEDLINE

DN 95405288 PubMed ID: 7674948
TI **Neurolysin**: purification and assays.
AU Checler F; Barelli H; Dauch P; Dive V; Vincent B; Vincent J P
CS Institut de Pharmacologie Moleculaire et Cellulaire, CNRS, Universite de Nice-Sophia Antipolis, Valbonne, France.
SO METHODS IN ENZYMOLOGY, (1995) 248 593-614.
Journal code: 0212271. ISSN: 0076-6879.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199510
ED Entered STN: 19951026
Last Updated on STN: 20000303
Entered Medline: 19951019
CT Check Tags: Animal; Male
Amino Acid Sequence
Brain: EN, enzymology
Metalloendopeptidases: AN, analysis
*Metalloendopeptidases: IP, isolation & purification
Mice
Molecular Sequence Data
Rats
Rats, Sprague-Dawley
Synaptic Membranes: EN, enzymology
Tissue Distribution
CN EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 31 OF 48 MEDLINE
AN 95405283 MEDLINE
DN 95405283 PubMed ID: 7674943
TI Thimet oligopeptidase and oligopeptidase M or **neurolysin**.
AU Barrett A J; Brown M A; Dando P M; Knight C G; McKie N; Rawlings N D; Serizawa A
CS Department of Biochemistry, Strangeways Research Laboratory, Cambridge, United Kingdom.
SO METHODS IN ENZYMOLOGY, (1995) 248 529-56.
Journal code: 0212271. ISSN: 0076-6879.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199510
ED Entered STN: 19951026
Last Updated on STN: 20000303
Entered Medline: 19951019
CT Check Tags: Animal; Human; Support, Non-U.S. Gov't
Amino Acid Sequence
Erythrocytes: EN, enzymology
Metalloendopeptidases: AN, analysis
Metalloendopeptidases: BL, blood
Metalloendopeptidases: CH, chemistry
*Metalloendopeptidases: ME, metabolism
Molecular Sequence Data
Rats
Sequence Alignment
CN EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.15 (thimet oligopeptidase); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 32 OF 48 MEDLINE

AN 95247711 MEDLINE
DN 95247711 PubMed ID: 7730308
TI Endopeptidase 24.16B. A new variant of endopeptidase 24.16.
AU Rodd D; Hersh L B
CS Department of Biochemistry, University of Kentucky, Lexington 40536-0084, USA.
NC DA 02243 (NIDA)
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 Apr 28) 270 (17) 10056-61. Journal code: 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199506
ED Entered STN: 19950608
Last Updated on STN: 20000303
Entered Medline: 19950601
AB A peptidase, isolated from rat testes, is inhibited by 1 mM o-phenanthroline, 1 microM N-(1-(R,S)-carboxyl-3-phenylpropyl)-Ala-Ala-Phe-p-aminobenzoate, and 6 mM Pro-Ile, properties similar to those ascribed to endopeptidase 24.16. The enzyme hydrolyzes dynorphin A-8, neurotensin 1-13, angiotensin I, and substance P. Kinetic analysis of a series of angiotensin I analogs showed that substitutions at P-1, P-1', or P-2' had little effect on Km or Kcat. Variation of peptide size with a series of dynorphin A peptides showed chain length to be significant. The peptidase cleaved dynorphin A-8 at both Leu5-Arg6 and Arg6-Arg7, and neurotensin 1-13 at Pro10-Tyr11 and Arg8-Arg9. In contrast, rat endopeptidase 24.16 cleaves dynorphin A-8 at Gly4-Leu5 and Leu5-Arg6, and neurotensin 1-13 only at Pro10-Tyr11. These findings, as well as the observation that endopeptidase 24.16 exhibits a considerably higher affinity for Pro-Ile, Ki = 90 microM, indicates the peptidase isolated in this study is related to, but distinct from, rat endopeptidase 24.16. We propose that this new endopeptidase be referred to as endopeptidase 24.16B, while the originally described enzyme be referred to as endopeptidase 24.16A.
CT Check Tags: Animal; Support, U.S. Gov't, P.H.S.
Amino Acid Sequence
Hydrolysis
Isoenzymes: IP, isolation & purification
*Isoenzymes: ME, metabolism
Kinetics
Metalloendopeptidases: IP, isolation & purification
*Metalloendopeptidases: ME, metabolism
Molecular Sequence Data
Rats
Substrate Specificity
CN 0 (Isoenzymes); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 33 OF 48 MEDLINE
AN 95138171 MEDLINE
DN 95138171 PubMed ID: 7836437
TI Characterization of a mitochondrial metallopeptidase reveals **neurolysin** as a homologue of thimet oligopeptidase.
AU Serizawa A; Dando P M; Barrett A J
CS Department of Biochemistry, Strangeways Research Laboratory, Cambridge, United Kingdom.

SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 Feb 3) 270 (5) 2092-8.
Journal code: 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199503
ED Entered STN: 19950314
Last Updated on STN: 20000303
Entered Medline: 19950302
AB We have isolated a metallopeptidase from rat liver. The peptidase is primarily located in the mitochondrial intermembrane space, where it interacts non-covalently with the inner membrane. The enzyme hydrolyzes oligopeptides, the largest substrate molecule found being dynorphin A1-17;
it has no action on proteins, and does not interact with alpha 2-macroglobulin, and can therefore be classified as an oligopeptidase. We
term the enzyme oligopeptidase M. Oligopeptidase M acts similarly to thimet oligopeptidase (EC 3.4.24.15) on bradykinin and several other peptides, but hydrolyzes neurotensin exclusively at the -Pro+Tyr- bond (the symbol + is used to indicate a scissile peptide bond) rather than the
-Arg+Arg- bond. The enzyme is inhibited by chelating agents and some thiol-blocking compounds, but differs from thimet oligopeptidase in not being activated by thiol compounds. The peptidase is inhibited by Pro-Ile, unlike thimet oligopeptidase, and the two enzymes are separable in chromatography on hydroxyapatite. The N-terminal amino acid sequence of rat mitochondrial oligopeptidase M contains 19 out of 20 residues identical with a segment of rabbit microsomal endopeptidase and 17 matching the corresponding segment of pig-soluble angiotensin II-binding protein. Moreover, the rat protein is recognized by a monoclonal antibody
against rabbit soluble angiotensin II-binding protein, all of which is consistent with these proteins being species variants of a single protein that is a homologue of thimet oligopeptidase. The biochemical properties of the mitochondrial oligopeptidase leave us in no doubt that it is **neurolysin** (EC 3.4.24.16), for which no sequence has previously been reported, and which has not been thought to be mitochondrial.
CT Check Tags: Animal; Support, Non-U.S. Gov't
Amino Acid Sequence
Hydrogen-Ion Concentration
Metalloendopeptidases: CH, chemistry
*Metalloendopeptidases: IP, isolation & purification
*Metalloendopeptidases: ME, metabolism
*Mitochondria, Liver: EN, enzymology
Molecular Sequence Data
Protease Inhibitors
Rats
Rats, Sprague-Dawley
Subcellular Fractions: EN, enzymology
Substrate Specificity
Sulfhydryl Reagents: PD, pharmacology
CN 0 (Protease Inhibitors); 0 (Sulfhydryl Reagents); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.15 (thimet oligopeptidase); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 34 OF 48 MEDLINE
AN 94305839 MEDLINE
DN 94305839 PubMed ID: 8032633

TI Role of endopeptidase 3.4.24.16 in the catabolism of neurotensin, in vivo,
in the vascularly perfused dog ileum.
AU Barelli H; Fox-Threlkeld J E; Dive V; Daniel E E; Vincent J P; Checler F
CS Institut de Pharmacologie Moleculaire et Cellulaire, UPR 411, CNRS Universite de Nice Sophia Antipolis, Valbonne, France.
SO BRITISH JOURNAL OF PHARMACOLOGY, (1994 May) 112 (1) 127-32.
Journal code: 7502536. ISSN: 0007-1188.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199408
ED Entered STN: 19940825
Last Updated on STN: 20000303
Entered Medline: 19940816
AB 1. The degradation of tritiated and unlabelled neurotensin (NT) following close intra-arterial infusion of the peptides in ileal segments of anaesthetized dogs was examined. 2. Intact NT and its catabolites recovered in the venous effluents were purified by chromatography on Sep-Pak columns followed by reverse-phase h.p.l.c. and identified by their retention times or by radioimmunoassay. 3. The half-life of neurotensin was estimated to be between 2 and 6 min. Four labelled catabolites, corresponding to free tyrosine, neurotensin (1-8), neurotensin (1-10) and neurotensin (1-11), were detected. 4. Neurotensin (1-11) was mainly generated by a phosphoramidon-sensitive cleavage, probably elicited by endopeptidase 24-11. 5. Two endopeptidase 3.4.24.16 inhibitors, phosphodiepryl 03 and the dipeptide Pro-Ile, dose-dependently potentiated the recovery of intact neurotensin. Furthermore, both agents inhibited the formation of neurotensin (1-10), the product that results from the hydrolysis of neurotensin by purified endopeptidase 3.4.24.16. In contrast, the endopeptidase 3.4.24.15 inhibitor Cpp-AAY-pAB neither protected neurotensin from degradation nor modified the production of neurotensin (1-10). 6. Our study is the first evidence to indicate that endopeptidase 3.4.24.16 contributes to the catabolism of neurotensin, in vivo, in the dog intestine.
CT Check Tags: Animal; Female; In Vitro; Male; Support, Non-U.S. Gov't
Amino Acid Sequence
Aminocaproic Acids: PD, pharmacology
Chromatography, High Pressure Liquid
Dogs
Ileum: DE, drug effects
Ileum: EN, enzymology
Ileum: ME, metabolism
Kinetics
Metalloendopeptidases: AI, antagonists & inhibitors
*Metalloendopeptidases: PH, physiology
Molecular Sequence Data
Muscle, Smooth: DE, drug effects
*Muscle, Smooth: EN, enzymology
Muscle, Smooth: ME, metabolism
Neurotensin: BI, biosynthesis
*Neurotensin: ME, metabolism
Neurotensin: PK, pharmacokinetics
Oligopeptides: PD, pharmacology
Peptide Fragments: BI, biosynthesis
Protease Inhibitors: PD, pharmacology
Radioimmunoassay

RN 130365-59-2 (N-(phenylethylphosphonyl)-glycyl-prolyl-aminohexanoic acid); 39379-15-2 (Neurotensin); 63524-00-5 (neurotensin (1-10))
CN 0 (Aminocaproic Acids); 0 (N-(1-carboxyl-3-phenylpropyl)alanyl-alanyl-tyrosyl-4-aminobenzoate); 0 (Oligopeptides); 0 (Peptide Fragments); 0 (Protease Inhibitors); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 35 OF 48 MEDLINE
AN 94087208 MEDLINE
DN 94087208 PubMed ID: 7903352
TI Endopeptidases 24.16 and 24.15 are responsible for the degradation of somatostatin, neurotensin, and other neuropeptides by cultivated rat cortical astrocytes.
AU Mentlein R; Dahms P
CS Anatomisches Institut, Universitat Kiel, F.R.G.
SO JOURNAL OF NEUROCHEMISTRY, (1994 Jan) 62 (1) 27-36.
Journal code: 2985190R. ISSN: 0022-3042.
CY United States.
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199401
ED Entered STN: 19940209
Last Updated on STN: 20000303
Entered Medline: 19940124
AB Several neuropeptides, including neurotensin, somatostatin, bradykinin, angiotensin II, substance P, and luteinizing hormone-releasing hormone but not vasopressin and oxytocin, were actively metabolized through proteolytic degradation by cultivated astrocytes obtained from rat cerebral cortex. Because phenanthroline was an effective degradation inhibitor, metalloproteases were responsible for neuropeptide fragmentation. Neurotensin was cleaved by astrocytes at the Pro10-Tyr11 and Arg8-Arg9 bonds, whereas somatostatin was cleaved at the Phe6-Phe7 and Thr10-Phe11 bonds. These cleavage sites have been found previously with endopeptidases 24.16 and 24.15 purified from rat brain. Addition of specific inhibitors of these proteases, the dipeptide Pro-Ile and N-[1-(RS)-carboxy-3-phenylpropyl]-Ala-Ala-Phe-4-aminobenzoate, significantly reduced the generation of the above neuropeptide fragments by astrocytes. The presence of endopeptidases 24.16 and 24.15 in homogenates of astrocytes could also be demonstrated by chromatographic separations of supernatant solubilized cell preparations. Proteolytic activity for neurotensin eluted after both gel and hydroxyapatite chromatography at the same positions as found for purified endopeptidase 24.16 or 24.15. In incubation experiments or in chromatographic separations no phosphoramidon-sensitive endopeptidase 24.11 (enkephalinase) or captopril-sensitive peptidyl dipeptidase A (angiotensin-converting enzyme) could be detected in cultivated astrocytes. Because astrocytes embrace the neuronal synapses where neuropeptides are released, we presume that the endopeptidases 24.16 and 24.15 on astrocytes are strategically located to contribute significantly to the inactivation of neurotensin, somatostatin, and other neuropeptides in the brain.
CT Check Tags: Animal; Support, Non-U.S. Gov't
Amino Acid Sequence
Animals, Newborn
*Astrocytes: EN, enzymology
Cells, Cultured
*Cerebral Cortex: EN, enzymology

Chromatography, High Pressure Liquid
Kinetics
*Metalloendopeptidases: ME, metabolism
Molecular Sequence Data
*Neuropeptides: ME, metabolism
*Neurotensin: ME, metabolism
Peptide Fragments: CH, chemistry
Peptide Fragments: IP, isolation & purification
Peptide Fragments: ME, metabolism
Protease Inhibitors: PD, pharmacology
Rats
Rats, Wistar
*Somatostatin: ME, metabolism
Substrate Specificity
RN 39379-15-2 (Neurotensin); 51110-01-1 (Somatostatin)
CN 0 (Neuropeptides); 0 (Peptide Fragments); 0 (Protease Inhibitors); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.15 (thimet oligopeptidase); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 36 OF 48 MEDLINE
AN 94040318 MEDLINE
DN 94040318 PubMed ID: 8224491
TI Recent advances on endopeptidase-3.4.24.16.
AU Checler F; Barelli H; Dauch P; Vincent B; Dive V; Beaudet A; Daniel E E; Fox-Threlkeld J E; Masuo Y; Vincent J P
CS Institut de Pharmacologie Moleculaire et Cellulaire, C.N.R.S. UPR 411-Universite de Nice Sophia Antipolis, Valbonne, France.
SO BIOCHEMICAL SOCIETY TRANSACTIONS, (1993 Aug) 21 (Pt 3) (3) 692-7. Journal code: 7506897. ISSN: 0300-5127.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199312
ED Entered STN: 19940117
Last Updated on STN: 20000303
Entered Medline: 19931209
CT Check Tags: Animal; Male; Support, Non-U.S. Gov't
Astrocytes: EN, enzymology
Base Sequence
*Brain: EN, enzymology
Cells, Cultured
Dogs
Fluorescent Dyes
Ileum: ME, metabolism
*Kidney: EN, enzymology
Kinetics
*Metalloendopeptidases: ME, metabolism
Molecular Sequence Data
Neprilysin: ME, metabolism
Neurotensin: ME, metabolism
Oligopeptides: ME, metabolism
Organ Specificity
Rats
Subcellular Fractions: EN, enzymology
Substrate Specificity
RN 39379-15-2 (Neurotensin)
CN 0 (Fluorescent Dyes); 0 (Oligopeptides); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.11 (Neprilysin); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 37 OF 48 MEDLINE
AN 93375416 MEDLINE
DN 93375416 PubMed ID: 8366428
TI Membrane-bound proteases involved in neuropeptide degradation in the brain.
AU Yokosawa H
CS Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan.
SO YAKUGAKU ZASSHI. JOURNAL OF THE PHARMACEUTICAL SOCIETY OF JAPAN, (1993 Jul) 113 (7) 504-14. Ref: 25
Journal code: 0413613. ISSN: 0031-6903.
CY Japan
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA Japanese
FS Priority Journals
EM 199310
ED Entered STN: 19931022
Last Updated on STN: 20000303
Entered Medline: 19931001
AB The action of neuropeptides at the synapse is terminated through enzymatic
degradation by membrane-bound proteases. We defined and purified membrane-bound proteases functioning at the initial stage of degradation of four neuropeptides. 1. Substance P-degrading endopeptidases isolated from the rat brain and pig striatum showed similar properties to those of endopeptidase-24.16 (**neurolysin**) except for cleavage sites of substance P. 2. LHRH fragment (1-5)-generating endopeptidases isolated from the neuroblastoma cells and rat brain showed similar properties to those of endopeptidase-24.15 (thimet oligopeptidase). 3. One of two dynorphin-degrading cysteine proteases isolated from neuroblastoma cells showed strict specificity toward the Arg-Arg residues. 4. Endopeptidase-24.11 (neprilysin) isolated from the rat brain was identified as a somatostatin-degrading enzyme.
CT Check Tags: Animal; Support, Non-U.S. Gov't
Brain: EN, enzymology
*Brain: ME, metabolism
English Abstract
Metalloendopeptidases: IP, isolation & purification
*Metalloendopeptidases: ME, metabolism
Neprilysin: IP, isolation & purification
Neprilysin: ME, metabolism
*Neuropeptides: ME, metabolism
Rats
Swine
*Synapses: ME, metabolism
CN 0 (Neuropeptides); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.- (substance P degrading enzyme); EC 3.4.24.11 (Neprilysin); EC 3.4.24.15 (thimet oligopeptidase); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 38 OF 48 MEDLINE
AN 93145978 MEDLINE
DN 93145978 PubMed ID: 8425555
TI Rat kidney endopeptidase 24.16. Purification, physico-chemical characteristics and differential specificity towards opiates, tachykinins and neurotensin-related peptides.
AU Barelli H; Vincent J P; Checler F
CS Institut de Pharmacologie Moleculaire et Cellulaire, Centre National de la

Recherche Scientifique, Universite Nice Sophia Antipolis, Valbonne, France.

SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (1993 Jan 15) 211 (1-2) 79-90.
Journal code: 0107600. ISSN: 0014-2956.
CY GERMANY: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199302
ED Entered STN: 19930312
Last Updated on STN: 20000303
Entered Medline: 19930226

AB Endopeptidase 24.16 was purified from rat kidney homogenate on the basis of its ability to generate the biologically inactive degradation products neurotensin (1-10) and neurotensin (11-13). On SDS gels of the proteins pooled after the last purification step, the enzyme appeared homogeneous and behaved as a 70-kDa monomer. The peptidase was not sensitive to specific inhibitors of aminopeptidases, pyroglutamyl aminopeptidase I, endopeptidase 24.11, endopeptidase 24.15, proline endopeptidase and angiotensin-converting enzyme but was potently inhibited by several metal chelators such as o-phenanthroline and EDTA and was blocked by divalent cations. The specificity of endopeptidase 24.16 towards peptides of the tachykinin, opioid and neurotensin families was examined by competition experiments of tritiated neurotensin hydrolysis as well as HPLC analysis. These results indicated that endopeptidase 24.16 could discriminate between peptides belonging to the same family. Neurotensin, Lys8-Asn9-neurotensin(8-13) and xenopsin were efficiently hydrolysed while neuromedin N and kinetensin underwent little if any proteolysis by the peptidase. Analogously, substance P and dynorphins (1-7) and (1-8) were readily proteolysed by endopeptidase 24.16 while neurokinin A, amphibian tachykinins and leucine or methionine enkephalins totally resisted degradation. By Triton X-114 phase separation, 15-20% of endopeptidase 24.16 partitioned in the detergent phase, indicating that renal endopeptidase 24.16 might exist in a genuine membrane-bound form. The equipotent solubilization of the enzyme by seven detergents of various critical miscellar concentrations confirmed the occurrence of a membrane-bound counterpart of endopeptidase 24.16. Furthermore, the absence of release elicited by phosphatidylinositol-specific phospholipase C suggested that the enzyme was not attached by a glycosyl-phosphatidylinositol anchor in the membrane of renal microvilli. Finally, endopeptidase 24.16 could not be released from these membranes upon trypsinolysis.

CT Check Tags: Animal; Support, Non-U.S. Gov't
Amino Acid Sequence
Cell Membrane: EN, enzymology
Kidney: EN, enzymology
Metalloendopeptidases: AI, antagonists & inhibitors
Metalloendopeptidases: CH, chemistry
*Metalloendopeptidases: IP, isolation & purification
Metalloendopeptidases: ME, metabolism
Microvilli: EN, enzymology
Molecular Sequence Data
Molecular Weight
Narcotics: ME, metabolism
Neuropeptides: ME, metabolism
Neurotensin: ME, metabolism
Phospholipase C: PD, pharmacology

Rats
Substrate Specificity
Tachykinins: ME, metabolism
Trypsin: PD, pharmacology
RN 39379-15-2 (Neurotensin)
CN 0 (Narcotics); 0 (Neuropeptides); 0 (Tachykinins); EC 3.1.4.3 (Phospholipase C); EC 3.4.21.4 (Trypsin); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 39 OF 48 MEDLINE
AN 93098580 MEDLINE
DN 93098580 PubMed ID: 1334389
TI Neurotensin receptor localization on neurons bearing the neurotensin-degrading enzyme endopeptidase 24-16.
AU Chabry J; Checler F; Vincent J P; Mazella J
CS Institut de Pharmacologie Moleculaire et Cellulaire du CNRS, Valbonne, France.
SO ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, (1992) 668 326-8.
Journal code: 7506858. ISSN: 0077-8923.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199301
ED Entered STN: 19930129
Last Updated on STN: 20000303
Entered Medline: 19930113
CT Check Tags: Animal
Brain: ME, metabolism
*Brain Chemistry
*Metalloendopeptidases: AN, analysis
Mice
Neurotensin: ME, metabolism
Receptors, Neurotensin
*Receptors, Neurotransmitter: AN, analysis
RN 39379-15-2 (Neurotensin)
CN 0 (Receptors, Neurotensin); 0 (Receptors, Neurotransmitter); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 40 OF 48 MEDLINE
AN 93075003 MEDLINE
DN 93075003 PubMed ID: 1332678
TI Potent inhibition of endopeptidase 24.16 and endopeptidase 24.15 by the phosphonamide peptide N-(phenylethylphosphonyl)-Gly-L-Pro-L-aminohexanoic acid.
AU Barelli H; Dive V; Yiotakis A; Vincent J P; Checler F
CS Institut de Pharmacologie Moleculaire et Cellulaire, UPR 411 du CNRS, Universite de Nice Sophia Antipolis, Valbonne, France.
SO BIOCHEMICAL JOURNAL, (1992 Oct 15) 287 (Pt 2) 621-5.
Journal code: 2984726R. ISSN: 0264-6021.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199212
ED Entered STN: 19930122
Last Updated on STN: 20000303
Entered Medline: 19921201
AB A phosphonamide peptide, N-(phenylethylphosphonyl)-Gly-L-Pro-L-aminohexanoic acid, previously shown to block Clostridium histolyticum

collagenases, was examined as a putative inhibitor of endopeptidase 24.16 and endopeptidase 24.15. Hydrolysis of two endopeptidase 24.16 substrates, i.e. 3-carboxy-7-methoxycoumarin (Mcc)-Pro-Leu-Gly-Pro-D-Lys-dinitrophenyl (Dnp) and neurotensin, were completely and dose-dependently inhibited by the phosphonamide inhibitor with KI values of 0.3 and 0.9 nM respectively. In addition, the phosphonamide peptide inhibited the hydrolysis of benzoyl (Bz)-Gly-Ala-Ala-Phe-(pAB) p-aminobenzoate and neurotensin by endopeptidase 24.15 with about a 10-fold lower potency (KI values of 5 and 7.5 nM respectively). The selectivity of this inhibitor towards several exo- and endo-peptidases belonging to the zinc-containing metallopeptidase family established that a 1 microM concentration of this inhibitor was unable to affect leucine aminopeptidase, carboxypeptidase A,
angiotensin-converting enzyme and endopeptidase 24.11. The present paper therefore reports on the first hydrophilic highly potent endopeptidase 24.16 inhibitor and describes the most potent inhibitory agent directed towards endopeptidase 24.15 developed to date. These tools should allow one to assess the contribution of endopeptidase 24.16 and endopeptidase 24.15 to the physiological inactivation of neurotensin as well as other neuropeptides.

CT Check Tags: Support, Non-U.S. Gov't
Amino Acid Sequence
*Aminocaproic Acids: PD, pharmacology
Carboxypeptidases: DE, drug effects
Carboxypeptidases: ME, metabolism
*Dipeptides: PD, pharmacology
Hydrolysis
Kinetics
Leucyl Aminopeptidase: DE, drug effects
Leucyl Aminopeptidase: ME, metabolism
*Metalloendopeptidases: AI, antagonists & inhibitors
Metalloendopeptidases: ME, metabolism
Molecular Sequence Data
Neprilysin: DE, drug effects
Neprilysin: ME, metabolism
Peptidyl-Dipeptidase A: DE, drug effects
Peptidyl-Dipeptidase A: ME, metabolism

RN 130365-59-2 (N-(phenylethylphosphonyl)-glycyl-prolyl-aminohexanoic acid)

CN 0 (Aminocaproic Acids); 0 (Dipeptides); EC 3.4.- (Carboxypeptidases); EC 3.4.11.1 (Leucyl Aminopeptidase); EC 3.4.15.1 (Peptidyl-Dipeptidase A); EC
3.4.17.1 (carboxypeptidase A); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.11 (Neprilysin); EC 3.4.24.15 (thimet oligopeptidase); EC 3.4.24.16
(**neurolysin**)

L6 ANSWER 41 OF 48 MEDLINE

AN 93019270 MEDLINE

DN 93019270 PubMed ID: 1402928

TI Endopeptidase 24-16 in murines: tissue distribution, cerebral regionalization, and ontogeny.

AU Dauch P; Masuo Y; Vincent J P; Checler F

CS Institut de Pharmacologie Moleculaire et Cellulaire, UPR 411 du CNRS, Universite de Nice Sophia Antipolis, Valbonne, France.

SO JOURNAL OF NEUROCHEMISTRY, (1992 Nov) 59 (5) 1862-7.
Journal code: 2985190R. ISSN: 0022-3042.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 199211
ED Entered STN: 19930122
Last Updated on STN: 20000303
Entered Medline: 19921120
AB The tissue distribution, cerebral regionalization, and ontogeny of endopeptidase 24-16 were established in murines by means of its quenched fluorimetric substrate, Mcc-Pro-Leu-Gly-Pro-D-Lys-Dnp, and its selective dipeptide blocker, Pro-Ile. Endopeptidase 24-16 was particularly abundant in the liver and kidney, and the lowest specific activity was detected in the heart. In the brain, a 16-fold difference in specific activity was observed between the poorest and the richest cerebral areas. Endopeptidase 24-16 appeared in high concentrations in the olfactory bulb and tubercule, cingulate cortex, medial striatum, and globus pallidus, and was particularly weak in the CA1, CA2, and CA3 parts of the hippocampal formation and in the cerebellum. Endopeptidase 24-16 content in thirteen thalamic nuclei indicated a rather homogeneous distribution. This homogeneity was not observed in the hypothalamus, where pronounced variations occurred between enriched zones such as suprachiasmatic and arcuate nuclei and relatively poor areas such as periventricular and supraoptic nuclei. Endopeptidase 24-16 appeared to be developmentally regulated in the mouse brain; it was already detected at the fetal stage, increased transiently after birth, then regularly declined until adulthood.
CT Check Tags: Animal; Male; Support, Non-U.S. Gov't
Amino Acid Sequence
Animals, Newborn: GD, growth & development
Animals, Newborn: ME, metabolism
*Brain: EN, enzymology
Brain: GD, growth & development
Embryo and Fetal Development
*Kidney: EN, enzymology
Kidney: GD, growth & development
*Liver: EN, enzymology
Liver: GD, growth & development
*Metalloendopeptidases: ME, metabolism
Metalloendopeptidases: PH, physiology
Mice
Molecular Sequence Data
Rats
Rats, Wistar
CN EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 42 OF 48 MEDLINE
AN 92380174 MEDLINE
DN 92380174 PubMed ID: 1355047
TI Purification of the main somatostatin-degrading proteases from rat and pig brains, their action on other neuropeptides, and their identification as endopeptidases 24.15 and 24.16.
AU Dahms P; Mentlein R
CS Universitat Kiel, Anatomisches Institut, Federal Republic of Germany.
SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (1992 Aug 15) 208 (1) 145-54.
Journal code: 0107600. ISSN: 0014-2956.
CY GERMANY: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199209

ED Entered STN: 19921018
Last Updated on STN: 20000303
Entered Medline: 19920929

AB The main somatostatin-degrading proteases were purified from rat and pig brain homogenates and characterized as thiol- and metal-dependent endoproteases. Two types of proteases with apparent native and subunit molecular masses of 70 kDa and 68 kDa could be differentiated in both species. Beside somatostatin, both hydrolyzed several other neuropeptides with chain lengths between 8 and 30 amino acid residues. Cleavage sites were generally similar or identical, but some clear exceptions were observed for enzymes from both species which could be used to differentiate between the two proteases. The 68-kDa protease cleaved somatostatin at three bonds (Asn5-Phe6, Phe6-Phe7 and Thr10-Phe11) and neurotensin only at the Arg8-Arg9 bond, whereas the 70-kDa protease digested somatostatin at only two bonds (Phe6-Phe7 and Thr10-Phe11) and neurotensin as well as acetylneurotensin-(8-13) additionally (pig protease) or almost exclusively (rat protease) at the Pro10-Tyr11 bond. Relative rates for the digestions of various peptides were, however, more dependent on the species than on the type of protease. Cleavage sites for angiotensin II, bradykinin, dynorphin, gonadoliberin and substance P were, apart from different rates, identical for both proteases. In both species the 68-kDa protease was found to be mainly, but not exclusively, soluble and not membrane-associated, whereas the inverse was detected for the 70-kDa protease. Based on distinct molecular and catalytic properties, the 68-kDa protease is supposed to be congruent with the endopeptidase 24.15 (EC 3.4.24.15), the 70-kDa protease with endopeptidase 24.16 (EC 3.4.24.16, neurotensin-degrading endopeptidase). This investigation demonstrates that both proteases hydrolyze various neuropeptides with similar cleavage sites, but with species-dependent activity. Species-independent distinctions are the exclusive action of endopeptidase 24.16 on acetylneurotensin-(8-13) and liberation of free Phe from somatostatin only by endopeptidase 24.15.

CT Check Tags: Animal; Support, Non-U.S. Gov't

Amino Acid Sequence
*Brain: EN, enzymology
Cell Membrane: EN, enzymology
Chromatography
Chromatography, Gel
Chromatography, Ion Exchange
Cytosol: EN, enzymology
Durapatite
Electrophoresis, Polyacrylamide Gel
Hydroxyapatites
Kinetics
*Metalloendopeptidases: IP, isolation & purification
*Metalloendopeptidases: ME, metabolism
Molecular Sequence Data
Molecular Weight
*Neuropeptides: ME, metabolism
Peptide Fragments: IP, isolation & purification
Rats
Rats, Inbred Strains
*Somatostatin: ME, metabolism
Substrate Specificity
Swine

RN 1306-06-5 (Dūrapatite); 51110-01-1 (Somatostatin)
CN 0 (Hydroxyapatites); 0 (Neuropeptides); 0 (Peptide Fragments); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.15 (thimet oligopeptidase); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 43 OF 48 MEDLINE
AN 92104144 MEDLINE
DN 92104144 PubMed ID: 1761032
TI Specific inhibition of endopeptidase 24.16 by dipeptides.
AU Dauch P; Vincent J P; Checler F
CS Institut de Pharmacologie Moleculaire et Cellulaire, Centre National de la Recherche Scientifique, Sophia Antipolis, Valbonne, France.
SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (1991 Dec 5) 202 (2) 269-76. Journal code: 0107600. ISSN: 0014-2956.
CY GERMANY: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199202
ED Entered STN: 19920302
Last Updated on STN: 20000303
Entered Medline: 19920211
AB The inhibitory effect of various dipeptides on the neurotensin-degrading metallopeptidase, endopeptidase 24.16, was examined. These dipeptides mimick the Pro10-Tyr11 bond of neurotensin that is hydrolyzed by endopeptidase 24.16. Among a series of Pro-Xaa dipeptides, the most potent inhibitory effect was elicited by Pro-Ile (Ki approximately 90 microM) with Pro-Ile greater than Pro-Met greater than Pro-Phe. All the Xaa-Tyr dipeptides were unable to inhibit endopeptidase 24.16. The effect of Pro-Ile on several purified peptidases was assessed by means of fluorigenic assays and HPLC analysis. A 5 mM concentration of Pro-Ile does not inhibit endopeptidase 24.11, endopeptidase 24.15, angiotensin-converting enzyme, proline endopeptidase, trypsin, leucine aminopeptidase, pyroglutamyl aminopeptidase I and carboxypeptidase B. The only enzyme that was affected by Pro-Ile was carboxypeptidase A, although it was with a 50-fold lower potency (Ki approximately 5 mM) than for endopeptidase 24.16. By means of fluorimetric substrates with a series of hydrolysing activities, we demonstrate that Pro-Ile can be used as a specific inhibitor of endopeptidase 24.16, even in a complex mixture of peptidase activities such as found in whole rat brain homogenate.
CT Check Tags: Animal; Male; Support, Non-U.S. Gov't
Amino Acid Sequence
Brain: EN, enzymology
Chromatography, High Pressure Liquid
*Dipeptides: PD, pharmacology
Hydrolysis
*Metalloendopeptidases: AI, antagonists & inhibitors
Metalloendopeptidases: IP, isolation & purification
Molecular Sequence Data
Neurotensin: ME, metabolism
Protease Inhibitors: IP, isolation & purification
Rats
Rats, Inbred Strains
RN 39379-15-2 (Neurotensin)
CN 0 (Dipeptides); 0 (Protease Inhibitors); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 44 OF 48 MEDLINE
AN 92082481 MEDLINE
DN 92082481 PubMed ID: 1747117
TI Fluorimetric assay of the neurotensin-degrading metalloendopeptidase, endopeptidase 24.16.
AU Dauch P; Barelli H; Vincent J P; Checler F
CS Institut de Pharmacologie Moleculaire et Cellulaire du CNRS, Valbonne, France.
SO BIOCHEMICAL JOURNAL, (1991 Dec 1) 280 (Pt 2) 421-6.
Journal code: 2984726R. ISSN: 0264-6021.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199201
ED Entered STN: 19920202
Last Updated on STN: 20000303
Entered Medline: 19920115
AB Mcc-Pro-Leu-Gly-Pro-D-Lys-Dnp (Mcc = 3-carboxy-7-methoxycoumarin; Dnp = dinitrophenyl), a quenched fluorimetric substrate originally designed as a probe to measure Pz-peptidase (also called endopeptidase 24.15), was examined as a putative substrate of the neurotensin-degrading neutral metalloendopeptidase, endopeptidase 24.16. During the purification of endopeptidase 24.16 the neurotensin(1-10) and neurotensin(11-13) formation due to this enzyme was coeluted with Mcc-Pro-Leu-Gly-Pro-D-Lys-Dnp-hydrolysing activity. By both fluorimetric and h.p.l.c. analyses, we observed that the latter activity was dose-dependently and completely abolished by neurotensin with an IC50 value (2.6 microM) that closely corresponds to the affinity of purified endopeptidase 24.16 for neurotensin (Km = 2.5 microM). Furthermore, Mcc-Pro-Leu-Gly-Pro-D-Lys-Dnp hydrolysis was inhibited by a series of dipeptides with a rank of order of potencies that parallels that observed in competition experiments of tritiated neurotensin hydrolysis by brain and intestinal endopeptidase 24.16. Altogether, these data clearly demonstrate that, in addition to Pz-peptidase, Mcc-Pro-Leu-Gly-Pro-D-Lys-Dnp also behaves as a substrate of endopeptidase 24.16, with a Km of about 26 microM. In addition, we show that, even in crude membrane preparations, Mcc-Pro-Leu-Gly-Pro-D-Lys-Dnp behaves as a useful tool to monitor and accurately quantify endopeptidase 24.16.
CT Check Tags: Animal; Support, Non-U.S. Gov't
Amino Acid Sequence
Brain: ME, metabolism
Chromatography, DEAE-Cellulose
Dipeptides: PD, pharmacology
*Fluorometry: MT, methods
Hydrolysis
Indicators and Reagents
Kinetics
*Metalloendopeptidases: AN, analysis
Metalloendopeptidases: ME, metabolism
Molecular Sequence Data
*Neurotensin: ME, metabolism
Oligopeptides: ME, metabolism
Rats

RN 127376-94-7 (7-methoxycoumarin-3-carboxylyl-prolyl-leucyl-glycyl-prolyl-lysyl-2,4-dinitrophenyl); 39379-15-2 (Neurotensin)
CN 0 (Dipeptides); 0 (Indicators and Reagents); 0 (Oligopeptides); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 45 OF 48 MEDLINE
AN 91291111 MEDLINE
DN 91291111 PubMed ID: 1905921
TI Purification and properties of a neurotensin-degrading endopeptidase from pig brain.
AU Millican P E; Kenny A J; Turner A J
CS Department of Biochemistry and Molecular Biology, University of Leeds, U.K.
SO BIOCHEMICAL JOURNAL, (1991 Jun 15) 276 (Pt 3) 583-91.
Journal code: 2984726R. ISSN: 0264-6021.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199108
ED Entered STN: 19910825
Last Updated on STN: 20000303
Entered Medline: 19910808
AB Neurotensin (NT) endopeptidase (EC 3.4.24.16) has been purified about 800-fold from pig brain by four sequential chromatographic steps depending on ion-exchange and hydrophobic interactions. Two types of preparation were studied: one from a Triton X-100-solubilized membrane fraction, and the other from the soluble fraction containing 90% or more of the total activity in the homogenate. NT endopeptidase activity was monitored by high-precision liquid chromatography of the two peptide products, characterized as NT-(1-10) and NT-(1-8), resulting from cleavage of the Pro10-Tyr11 and Arg8-Arg9 bonds respectively. As purification proceeded, from both membranes and cytosol, the yield of the two products achieved a constant ratio of 5:1 and this ratio was reproduced in repeated purifications. However, a distinct peptidase which hydrolysed exclusively at the Arg8-Arg9 bond was partially resolved from NT endopeptidase by chromatography on hydroxyapatite, and this activity was further purified and assigned to endopeptidase-24.15 (EC 3.4.24.15). SDS/PAGE of both preparations of neurotensin endopeptidase revealed a major band of apparent Mr 75000, and treatment of the membrane-associated form with N-Glycanase gave no evidence that the enzyme was a glycoprotein. The membrane-associated and cytosol forms of NT endopeptidase activities, monitored for both NT-(1-10) and NT-(1-8) products, were compared in their responses to 1,10-phenanthroline, EDTA, dithiothreitol (DTT) and some synthetic site-directed inhibitors of endopeptidase-24.15 or peptidyl dipeptidase A. The effects revealed no significant differences between the two preparations, nor did the reagents discriminate between the activities generating the two NT fragments. The partially purified form of endopeptidase-24.15 was also included in this comparison: while some responses were similar, this peptidase was distinguishable in its activation by DTT and its relative resistance to inhibition by EDTA. Both forms of NT endopeptidase were found to hydrolyse other substrates, including Boc-Phe-Ala-Ala-Phe-4-aminobenzoate, bradykinin and substance P (these at faster rates than neurotensin), as well as dynorphin A-(1-8) and luliberin. The bonds hydrolysed in these neuropeptides, as well as in

angiotensins I and II and alpha-neoendorphin, were defined. These studies
confirm that NT endopeptidase is distinct from endopeptidase-24.15. They
further show that the former is a soluble enzyme, not an integral membrane
protein, that it is not peptide-specific and that it might be more
appropriately named. enzyme, not an integral membrane protein, that it is
not peptide-specific and

CT Check Tags: Animal; Support, Non-U.S. Gov't
Amino Acid Sequence
Binding Sites
Brain: DE, drug effects
*Brain: EN, enzymology
Cell Membrane: DE, drug effects
Cell Membrane: EN, enzymology
Chromatography, Liquid
Cytosol: DE, drug effects
Cytosol: EN, enzymology
Dithiothreitol: PD, pharmacology
Edetic Acid: PD, pharmacology
Electrophoresis, Polyacrylamide Gel
Hydrolysis
Metalloendopeptidases: CH, chemistry
*Metalloendopeptidases: IP, isolation & purification
Molecular Sequence Data
Neurotensin: AI, antagonists & inhibitors
*Neurotensin: ME, metabolism
Peptides: CS, chemical synthesis
Phenanthrolines: PD, pharmacology
Substrate Specificity
Swine

RN 3483-12-3 (Dithiothreitol); 39379-15-2 (Neurotensin); 60-00-4 (Edetic Acid); 66-71-7 (1,10-phenanthroline)

CN 0 (Peptides); 0 (Phenanthrolines); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.15 (thimet oligopeptidase); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 46 OF 48 MEDLINE

AN 91101048 MEDLINE

DN 91101048 PubMed ID: 2176676

TI Colocalization of neurotensin receptors and of the neurotensin-degrading enzyme endopeptidase 24-16 in primary cultures of neurons.

AU Chabry J; Checler F; Vincent J P; Mazella J

CS Institut de Pharmacologie Moleculaire et Cellulaire Centre National de la Recherche Scientifique, Valbonne, France.

SO JOURNAL OF NEUROSCIENCE, (1990 Dec) 10 (12) 3916-21.
Journal code: 8102140. ISSN: 0270-6474.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 199102

ED Entered STN: 19910329
Last Updated on STN: 20000303
Entered Medline: 19910220

AB This paper compares the localization of neurotensin receptors and of endopeptidase 24-16, a peptidase likely involved in the inactivation of neurotensin in primary cultures of neurons. Neurotensin binding sites were radiolabeled with 125I-Tyr3-neurotensin, whereas endopeptidase 24-16 was stained by immunohistochemical techniques using a monospecific polyclonal antibody. Endopeptidase 24-16 is present in 80-85% of the

nondifferentiated neurons. The proportion of immunoreactive neurons decreased during maturation to reach 35-40% after 4-8 d of culture. By contrast, neurotensin receptors were not detectable in nondifferentiated cells and appear during maturation. Specific 125I-Tyr3-neurotensin labeling is maximal after 4 d of culture and is located on about 10% of differentiated neurons. Double-labeling experiments show that about 90% of cortical, hypothalamic, and mesencephalic neurons bearing the neurotensin receptor also contained endopeptidase 24-16, supporting the hypothesis that one of the functions of endopeptidase 24-16 is the physiological inactivation of neurotensin. However, the presence of endopeptidase 24-16 on numerous neurons that do not contain neurotensin receptors also suggests that the enzyme could be involved in the degradation and/or maturation of other neuropeptides.

CT Check Tags: Animal; Support, Non-U.S. Gov't
Brain: ME, metabolism
Cell Differentiation
Cells, Cultured
Iodine Radioisotopes
*Metalloendopeptidases: ME, metabolism
Metalloendopeptidases: PH, physiology
Mice
Monoiodotyrosine: ME, metabolism
Neurons: EN, enzymology
*Neurons: ME, metabolism
Neuropeptides: ME, metabolism
*Neurotensin: ME, metabolism
Receptors, Neurotensin
*Receptors, Neurotransmitter: ME, metabolism

RN 29592-76-5 (Monoiodotyrosine); 39379-15-2 (Neurotensin)

CN 0 (Iodine Radioisotopes); 0 (Neuropeptides); 0 (Receptors, Neurotensin); 0 (Receptors, Neurotransmitter); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 47 OF 48 MEDLINE

AN 90115551 MEDLINE

DN 90115551 PubMed ID: 2575247

TI Neuropeptide-hydrolysing activities in synaptosomal fractions from dog ileum myenteric, deep muscular and submucous plexi. Their participation in neurotensin inactivation.

AU Barelli H; Ahmad S; Kostka P; Fox J A; Daniel E E; Vincent J P; Checler F

CS Centre National de la Recherche Scientifique, Faculte des Sciences, Nice, France.

SO PEPTIDES, (1989 Sep-Oct) 10 (5) 1055-61.
Journal code: 8008690. ISSN: 0196-9781.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 199002

ED Entered STN: 19900328
Last Updated on STN: 20000303
Entered Medline: 19900216

AB The mapping of neuropeptidases in synaptosomal fractions prepared from dog ileum myenteric, deep muscular and submucous plexus was established by means of fluorigenic substrates and specific inhibitors. Endopeptidase 24.11, angiotensin-converting enzyme and aminopeptidases were found in all

tissues, the highest amounts being recovered in the submucous preparation.
Post-proline dipeptidyl aminopeptidase was obtained in high quantities whatever the tissue source while proline endopeptidase was detected in low
amounts and pyroglutamyl-peptide hydrolase was never detectable. The above peptidases were examined for their putative participation in the inactivation of neurotensin by monitoring the effect of specific inhibitors on the formation of the metabolites of labeled neurotensin separated by HPLC. Endopeptidases 24.11, 24.15 and 24.16 were respectively responsible for the formation of neurotensin(1-11), neurotensin(1-8) and neurotensin(1-10) that are devoid of biological activity. The secondary attacks occurring on neurotensin degradation products were the following: cleavage of neurotensin(1-10) into neurotensin(1-8) by angiotensin-converting enzyme; conversion of neurotensin(9-13) into neurotensin(11-13) by post-proline dipeptidyl aminopeptidase; hydrolysis of neurotensin(11-13) into free tyrosine by aminopeptidase(s).
CT Check Tags: Animal; Support, Non-U.S. Gov't
Aminopeptidases: ME, metabolism
Chromatography, High Pressure Liquid
Dogs
Endopeptidases: ME, metabolism
Fluorometry
Hydrolysis
*Ileum: IR, innervation
Membranes: EN, enzymology
Metalloendopeptidases: ME, metabolism
*Myenteric Plexus: EN, enzymology
Neprilysin: ME, metabolism
*Neuropeptides: ME, metabolism
*Neurotensin: ME, metabolism
Peptidyl-Dipeptidase A: ME, metabolism
Pyroglutamyl-Peptidase I: ME, metabolism
*Submucous Plexus: EN, enzymology
*Synaptosomes: EN, enzymology
RN 39379-15-2 (Neurotensin)
CN 0 (Neuropeptides); EC 3.4.- (Endopeptidases); EC 3.4.11 (Aminopeptidases);
EC 3.4.15.1 (Peptidyl-Dipeptidase A); EC 3.4.19.3 (Pyroglutamyl-Peptidase I); EC 3.4.21.26 (prolyl oligopeptidase); EC 3.4.24 (Metalloendopeptidases); EC 3.4.24.11 (Neprilysin); EC 3.4.24.15 (thimet oligopeptidase); EC 3.4.24.16 (**neurolysin**)

L6 ANSWER 48 OF 48 MEDLINE
AN 59078185 MEDLINE
DN 59078185
TI [Effect of **neurolysin** on the mechanism of immunobiological protection].
Die Wirkung der Neurolysine auf den Mechanismus des immunbiologischen Schutzes. II. Der Einfluss der Neurolysine auf die Phagozytose in vivo.
AU MARKOV W; VULCHANOV V H; ILIEWA W
SO Zschr. Immunforsch, (1959 Mar) 117 (3) 177-89.
DT Journal
LA German
FS OLDMEDLINE
OS CLML5936-22472-271-409
EM 195912
ED Entered STN: 20000825
Last Updated on STN: 20000825

ST immune serums - effects; phagocytosis - effect of drugs on
RN 89957-38-0 (IMMUNE SERUMS)